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(54) Title: OSTEOPROTEGERIN

(57) Abstract

The present invention discloses a secreted polypeptide, termed osteoprotegerin, which is a member of the tumor necrosis factor receptor superfamily and is involved in the regulation of bone metabolism. Also disclosed are nucleic acids encoding osteoprotegerin, polypeptides, recombinant vectors and host cells for expression, antibodies which bind OPG, and pharmaceutical compositions. The polypeptides are used to treat bone diseases characterized by increased resorption such as osteoporosis.

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OSTEOPROTEGERIN

Field of the Invention

The invention relates generally to polypeptides involved in the regulation of bone metabolism. More particularly, the invention relates to a novel polypeptide, termed osteoprotegerin, which is a member of the tumor necrosis factor receptor superfamily. The polypeptide is used to treat bone diseases characterized by increased bone loss such as osteoporosis.

Background of the Invention

Polypeptide growth factors and cytokines are secreted factors which signal a wide variety of changes in cell growth, differentiation, and metabolism, by specifically binding to discrete, surface bound receptors. As a class of proteins, receptors vary in their structure and mode of signal transduction. They are characterized by having an extracellular domain that is involved in ligand binding, and cytoplasmic domain which transmits an appropriate intracellular signal. Receptor expression patterns ultimately determine which cells will respond to a given ligand, while the structure of a given receptor dictates the cellular response induced by ligand binding. Receptors have been shown to transmit intracellular signals via their cytoplasmic domains by activating protein tyrosine, or protein serine/threonine phosphorylation (e.g., platelet derived growth factor receptor (PDGFR) or transforming growth factor-β receptor-I (TGFβR-I), by stimulating G-protein activation (e.g., β-adrenergic receptor), and by modulating associations with cytoplasmic signal

transducing proteins (e.g., TNFR-1 and Fas/APO) (Heldin, Cell 80, 213-223 (1995)).

The tumor necrosis factor receptor (TNFR) superfamily is a group of type I transmembrane proteins which share a conserved cysteine-rich motif which is repeated three to six times in the extracellular domain (Smith, et al. Cell 76, 953-962 (1994)). Collectively, these repeat units form the ligand binding domains of these receptors (Chen et al., Chemistry 270, 2874-2878 (1995)). The ligands for these receptors are a structurally related group of proteins homologous to TNFα. (Goeddel et al. Cold Spring Harbor Symp. Quart. Biol. 51, 597-609 (1986); Nagata et al. Science 267, 1449-1456 (1995)). TNFα binds to distinct, but closely related receptors, TNFR-1 and TNFR-2. TNFα produces a variety of biological responses in receptor bearing cells, including, proliferation, differentiation, and cytotoxicity and apoptosis (Beutler et al. Ann. Rev. Biochem. 57, 505-518 (1988)).

TNFα is believed to mediate acute and chronic inflammatory responses (Beutler et al. Ann. Rev. Biochem. 57, 505-508 (1988)). Systemic delivery of TNFα induces toxic shock and widespread tissue necrosis. Because of this, TNFα may be responsible for the severe morbidity and mortality associated with a variety of infectious diseases, including sepsis. Mutations in FasL, the ligand for the TNFR-related receptor Fas/APO (Suda et al. Cell 75, 1169-1178 (1993)), is associated with autoimmunity (Fisher et al. Cell 81, 935-946 (1995)), while overproduction of FasL may be implicated in drug-induced hepatitis. Thus, ligands to the various TNFR-related proteins often mediate the serious effects of many disease states, which suggests that agents that

neutralize the activity of these ligands would have therapeutic value. Soluble TNFR-1 receptors, and antibodies that bind TNFα, have been tested for their ability to neutralize systemic TNFα (Loetscher et al. Cancer Cells 3(6), 221-226 (1991)). A naturally occurring form of a secreted TNFR-1 mRNA was recently cloned, and its product tested for its ability to neutralize TNFα activity *in vitro* and *in vivo* (Kohno et al. PNAS USA 87, 8331-8335 (1990)). The ability of this protein to neutralize TNFα suggests that soluble TNF receptors function to bind and clear TNF thereby blocking the cytotoxic effects on TNFR- bearing cells.

An object of the invention to identify new members of the TNFR super family. It is anticipated that new family members may be transmembrane proteins or soluble forms thereof comprising extracellular domains and lacking transmembrane and cytoplasmic domains. We have identified a new member of the TNFR superfamily which encodes a secreted protein that is closely related to TNFR-2. By analogy to soluble TNFR-1, the TNFR-2 related protein may negatively regulate the activity of its ligand, and thus may be useful in the treatment of certain human diseases.

Summary of the Invention

A novel member of the tumor necrosis factor receptor (TNFR) superfamily has been identified from a fetal rat intestinal cDNA library. A full-length cDNA clone was obtained and sequenced. Expression of the rat cDNA in a transgenic mouse revealed a marked increase in bones density, particularly in long bones, pelvic bone and vertebrae. The polypeptide encoded by the cDNA is termed Osteprotegerin (OPG) and plays a role in promoting bone accumulation.

The invention provides for nucleic acids encoding a polypeptide having at least one of the biological activities of OPG. Nucleic acids which hybridize to nucleic acids encoding mouse, rat or human OPG as shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO: 122), and 9C-9D (SEQ ID NO: 124) are also provided. Preferably, OPG is mammalian OPG and more preferably is human OPG. Recombinant vectors and host cells expressing OPG are also encompassed as are methods of producing recombinant OPG. Antibodies or fragments thereof which specifically bind the polypeptide are also disclosed.

Methods of treating bone diseases are also provided by the invention. The polypeptides are useful for preventing bone resorption and may be used to treat any condition resulting in bone loss such as osteoporosis, hypercalcemia, Paget's disease of bone, and bone loss due to rheumatoid arthritis or osteomyelitis, and the like. Bone diseases may also be treated with anti-sense or gene therapy using nucleic acids of the invention. Pharmaceutical compositions comprising OPG nucleic acids and polypeptides are also encompassed.

Description of the Figures

Figure 1. A. FASTA analysis of novel EST LORF. Shown is the deduced FRI-1 amino acid sequence aligned to the human TNFR-2 sequence. B. Profile analysis of the novel EST LORF shown is the deduced FRI-1 amino acid sequence aligned to the TNFR-profile. C. Structural view of TNFR superfamily indicating region which is homologous to the novel FRI-1.

Figure 2. Structure and sequence of full length rat OPG gene, a novel member of the TNFR superfamily. A. Map of pMOB-B1.1 insert. Box indicates position of LORF within the cDNA sequence (bold line). Black box indicates signal peptide, and gray ellipses indicate position of cysteine-rich repeat sequences. B, C. Nucleic acid and protein sequence of the Rat OPG cDNA. The predicted signal peptide is underlined, and potential sites of N-linked glycosylation are indicated in bold, underlined letters. D, E. Pileup sequence comparison (Wisconsin GCG Package, Version 8.1) of OPG with other members of the TNFR superfamily, fas (SEQ ID NO:128); tnfr1 (SEQ ID NO: 129); sfu-t2 (SEQ ID NO:130); tnfr2 (SEQ ID NO:131); cd40 (SEQ ID NO:132); osteo (SEQ ID NO:133); ngfr (SEQ ID NO:134); ox40 (SEQ ID NO:135); 41bb (SEQ ID NO:136).

Figure 3. PepPlot analysis (Wisconsin GCG Package, Version 8.1) of the predicted rat OPG protein sequence. A. Schematic representation of rat OPG showing hydrophobic (up) and hydrophilic (down) amino acids. Also shown are basic (up) and acidic (down) amino acids. B. Display of amino acid residues that are beta-sheet forming (up) and beta-sheet breaking down) as defined by Chou and Fasman (Adv. Enz. 47, 45-147 (1948)). C. Display of propensity measures for alpha-helix and beta-sheet (Chou and Fasman, ibid). Curves above 1.00 show propensity for alpha-helix or beta-sheet structure. Structure may terminate in regions of protein where curves drop below 1.00. D. Display of residues that are alpha-forming (up) or alpha-breaking (down). E. Display of portions of the protein sequence that resemble sequences typically found at the amino end of alpha and beta structures (Chou and Fasman, ibid). F. Display of

portions of the protein sequence that resemble sequences typically found at the carboxyl end of alpha and beta structures (Chou and Fasman, ibid). G. Display of portions of the proteins sequence typically found in turns (Chou and Fasman, ibid) H. Display of the helical hydrophobic moment (Eisenberg et al. Proc. Natl. Acad. Sci. USA 81, 140-144 (1984)) at each position in the sequence. I. Display of average hydrophathy based upon Kyte and Doolittle (J. Mol. Biol. 157, 105-132 (1982)) and Goldman et al. (reviewed in Ann. Rev. Biophys. Biophys. Chem. 15, 321-353 (1986)).

Figure 4. mRNA expression patterns for the OPG cDNA in human tissues. Northern blots were probed with a 32P-labeled rat cDNA insert (A, left two panels), or with the human cDNA insert (B, right panel).

Figure 5. Creation of transgenic mice expressing the OPG cDNA in hepatocytes. Northern blot expression of HE-OPG transgene in mouse liver.

Figure 6. Increase in bone density in OPG transgenic mice. Panel A-F. Control Mice. G-J, OPG expressing mice. At necropsy, all animals were radiographed and photographs prepared. In A-F, the radiographs of the control animals and the one transgenic non-expressor (#28) are shown. Note that the bones have a clearly defined cortex and a lucent central marrow cavity. In contrast, the OPG (G-J) animals have a poorly defined cortex and increased density in the marrow zone.

Figure 7. Increase in trabecular bone in OPG transgenic mice. A-D. Representative photomicrographs of bones from control animals. In A and B, low (4X, 10X) power

images of the femurs are shown (Masson Trichrome stain). Stains for tartrate resistant acid phosphatase (TRAP) demonstrate osteoclasts (see arrows) both resorbing cartilage (C) and trabecular bone (D). Note the flattened appearance of osteoclasts on trabecular bone. E-H. Representative photomicrographs of bones from OPG-expressing animals. In E and F, low (4X, 10X) power images of the femurs are shown (Masson Trichrome stain). The clear region is the growth plate cartilage, blue stained area is bone, and the red area is marrow. Note that in contrast to the controls, the trabecular bone has not been resorbed resulting in the absence of the usual marrow cavity. Also, the resulting trabeculae have a variegated appearance with blue and clear areas. The clear areas are remnants of growth plate cartilage that have never been remodelled. Based on TRAP stains, these animals do have osteoclasts (see arrows) at the growth plate (G), which may be reduced in number. However, the surfaces of the trabeculae away from the growth plate are virtually devoid of osteoclasts (H), a finding that stands in direct contrast with the control animals (see D).

Figure 8. HE-OPG expressors do not have a defect in monocyte-macrophage development. One cause for osteopetrosis in mice is defective M-CSF production due to a point mutation in the M-CSF gene. This results in a marked deficit of circulating and tissue based macrophages. The peripheral blood of OPG expressors contained monocytes as assessed by H1E analysis. To affirm the presence of tissue macrophages, immnohistochemistry was performed using F480 antibodies, which recognize a cell surface antigen on murine macrophages. A and C show low power (4X)

photomicrographs of the spleens from normal and CR1 overexpressors. Note that both animals have numerous F480 positive cells. Monocyte-macrophages were also present in the marrow of normal (B) and HE-OPG overexpressors (D) (40X).

Figure 9. Structure and sequence of mouse and human OPG cDNA clones. A, B. Mouse cDNA and protein sequence. C, D. Human cDNA and protein sequence. The predicted signal peptides are underlined, and potential sites of N-linked glycosylation are indicated in bold. E, F. Sequence alignment and comparison of rat, mouse and human OPG amino acid sequences.

Figure 10. Comparison of conserved sequences in extracellular domain of TNFR-1 and human OPG. PrettyPlot (Wisconsin GCG Package, Version 8.1) of the TNFR1 and OPG alignment described in example 6. Top line, human TNFR1 sequences encoding domains 1-4. Bottom line, human OPG sequences encoding domains 1-4. Conserved residues are highlighted by rectangular boxes.

Figure 11. Three-dimensional representation of human OPG. Side-view of the Molescript display of the predicted 3-dimensional structure of human OPG residues 25 through 163, (wide line), co-crystallized with human TNFβ (thin line). As a reference for orientation, the bold arrows along the OPG polypeptide backbone are pointing in the N-terminal to C-terminal direction. The location of individual cysteine residue side chains are inserted along the polypeptide backbone to help demonstrate the separate cysteine-rich domains. The TNFβ molecule is aligned as described by Banner et al. (1993).

Figure 12. Structure of OPG cysteine-rich domains. Alignment of the human (top line SEQ ID NO:136) and mouse (bottom line) OPG amino acid sequences highlighting the predicted domain structure of OPG. The polypeptide is divided into two halves; the N-terminus (A), and C-terminus (B). The N-terminal half is predicted to contain four cysteine rich domains (labeled 1-4). The predicted intrachain disulfide bonds are indicated by bold lines, labeled "SS1", "SS2", or "SS3". Tyrosine 28 and histidine 75 (underlined) are predicted to form an ionic interaction. Those amino acids predicted to interact with an OPG ligand are indicated by bold dots above the appropriate residue. The cysteine residues located in the C-terminal half of OPG are indicated by rectangular boxes.

Figure 13. Expression and secretion of full length and truncated mouse OPG-Fc fusion proteins. A. Map indicating points of fusion to the human IgG1 Fc domain are indicated by arrowheads. B. Silver stain of a SDS-polyacrylamide gel of conditioned media obtained from cells expressing either Fl.Fc (Full length OPG fused to Fc at Leucine 401) or CT.Fc (Carboxy-terminal truncated OPG fused to Fc at threonine 180) fusion protein expression vectors. Lane 1, parent pCEP4 expression vector cell line; Lane 2, Fl.Fc vector cell line; Lane 3, CT.Fc vector cell line. C. Western blot of conditioned media obtained from Fl.Fc and CT.Fc fusion protein expression vectors probed with anti-human IgG1 Fc domain (Pierce). Lane 1, parent pCEP4 expression vector cell line; Lane 2, Fl.Fc vector cell line; Lane 3, CT.Fc vector cell line.

Figure 14. Expression of human OPG in E. coli. A. Construction of a bacterial expression vector. The LORF of the human OPG gene was amplified by PCR, then joined to a oligonucleotide linker fragment (top strand is SEQ ID NO:137; bottom strand is SEQ ID NO:127), and ligated into pAMG21 vector DNA. The resulting vector is capable of expressing OPG residues 32-401 linked to a N-terminal methionine residue. B SDS-PAGE analysis of uninduced and induced bacterial harboring the pAMG21-human OPG -32-401 plasmid. Lane 1, MW standards; lane 2, uninduced bacteria; lane 3, 30°C induction; lane 4, 37°C induction; lane 5, whole cell lysate from 37°C induction; lane 6, soluble fraction of whole cell lysate; lane 7, insoluble fraction of whole cell lysate; lane 8, purified inclusion bodies obtained from whole cell lysate.

Figure 15. Analysis of recombinant murine OPG produced in CHO cells by SDS-PAGE and western blotting. An equal amount of CHO conditioned media was applied to each lane shown, and was prepared by treatment with either reducing sample buffer (left lane), or non-reducing sample buffer (right lane). After electrophoresis, the resolved proteins were transferred to a nylon membrane, then probed with anti-OPG antibodies. The relative positions of the 55 kd monomeric and 100 kd dimeric forms of OPG are indicated by arrowheads.

Figure 16. Pulse-chase analysis of recombinant murine OPG produced in CHO cells. CHO cells were pulse-labeled with ^{35}S-methionine/cysteine, then chased for the indicated time. Metabolically labeled cultures were separated into both conditioned media and cells, and detergent extracts were prepared from each, clarifi d,

then immunoprecipitated with anti-OPG antibodies. The immunoprecipitates were the resolved by SDS-PAGE, and exposed to film. Top left and right panels; samples analyzed under non-reducing conditions. Lower left and right panels; samples analyzed under reducing conditions. Top and bottom left panels; Cell extracts. Top and bottom right panels; Conditioned media extracts. The relative mobility of the 55 kd monomeric and 100 kd dimeric forms of OPG are indicated by arrowheads.

Figure 17. Expression of OPG in the CTLL-2 cell line. Serum-free conditioned media from CTLL-2 cells and CHO-mu OPG [1-401] transfected cells was prepared, concentrated, then analyzed by non-reducing SDS-PAGE and western blotting. Left lane; CTLL-2 conditioned media. Right lane; CHO-muOPG conditioned media. The relative mobility of the 55 kd monomeric and 100 kd dimeric forms of OPG are indicated by arrowheads.

Figure 18. Detection of OPG expression in serum samples and liver extracts obtained from control and OPG transgenic mice. Transgenic mice were constructed as described in Example 4. OPG expression was visualized after SDS-PAGE followed by Western blotting using anti-OPG antibodies.

Figure 19. Effects of huOPG [22-401]-Fc fusion protein on osteoclast formation <u>in vitro</u>. The osteoclast forming assay was performed as described in Example 11A in the absence (control) or presence of the indicated amounts of huOPG [22-401]-Fc fusion. Osteoclast formation was visualized by histochemical staining for tartrate acid phosphatase (TRAP).). A. OPG added to 100 ng/ml. D. OPG added to 0.1 ng/ml. E. OPG added to

0.01 ng/ml. F. OPG added to 0.001 ng/ml. G. Control. No OPG added.

Figure 20. Decrease in osteoclast culture TRAP activity with increasing amounts of OPG. Indicated concentrations of huOPG [22-401]-Fc fusion protein were added to osteoclast forming assay and TRAP activity quantitated as described in Example 11A.

Figure 21. Effect of OPG on a terminal stage of osteoclast differentiation. huOPG [22-401]-Fc fusion was added to the osteoclast forming assay during the intermediate stage of osteoclast maturation (days 5-6; OPG-CTL) or during the terminal stage of osteoclast maturation (days 7-15; CTL-OPG). TRAP activity was quantitated and compared with the activity observed in the absence of OPG (CTL-CTL) in the presence of OPG throughout (OPG-OPG).

Figure 22. Effects of IL-1β, IL-1α and OPG on blood ionized calcium in mice. Levels of blood ionized calcium were monitored after injection of IL-1β alone, IL-1α alone, IL-1β plus muOPG [22-401]-Fc, IL-1α plus MuOPG [22-401]-Fc, and muOPG [22-401]-Fc alone. Control mice received injections of phosphate buffered saline (PBS) only. IL-1B experiment shown in A; IL-1α experiment shown in B.

Figure 23. Effects of OPG on calvarial osteoclasts in control and IL1-treated mice. Histological methods for analyzing mice calvarial bone samples are described in Example 11B. Arrows indicate osteoclasts present in day 2-treated mice. Calvarial samples of mice receiving four PBS injections daily (A), one injection of IL-1 and

three injections of PBS daily (B), one injection of PBS and three injections of OPG daily (C), one injection of IL-1 and three injections of OPG daily.

Figure 24. Radiographic analysis of bone accumulation in marrow cavity of normal mice. Mice were injected subcutaneously with saline (A) or muOPG [22-401]-Fc fusion (5mg/kg/d) for 14 days (B) and bone density determined as described in Example 11C.

Figure 25. Histomorphometric analysis of bone accumulation in marrow cavity of normal mice. Injection experiments and bone histology performed as described in Example 11C.

Figure 26. Histology analysis of bone accumulation in marrow cavity of normal mice. Injection experiments and bone histology performed as described in Example 11C. A. Saline injection B. Injection of muOPG [22-401]-Fc fusion.

Figure 27. Activity of OPG administered to ovariectomized rats. In this two week experiment the trend to reduced bone density appears to be blocked by OPG or other anti-resorptive therapies. DEXA measurements were taken at time of ovariectomy and at week 1 and week 2 of treatment. The results are expressed as % change from the initial bone density (Mean +/- SEM).

Figure 28. Bone density in the femoral metaphysis, measured by histomorphometric methods, tends to be lower in ovariectomized rats (OVX) than sham operated animals (SHAM) 17 days following ovariectomy. This effect was

blocked by OPG-Fc, with OPG-Fc treated ovariectomized rats (OVX+OPG) having significantly higher bone density than vehicle treated ovariectomized rats (OVX). (Mean +/- SEM).

Detailed Description of the Invention

A novel member of the tumor necrosis factor receptor (TNFR) superfamily was identified as an expressed sequence tag (EST) isolated from a fetal rat intestinal cDNA library . The structures of the full-length rat cDNA clones and the corresponding mouse and human cDNA clones were determined as described in Examples 1 and 6. The rat, mouse and human genes are shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124), respectively. All three sequences showed strong similarity to the extracellular domains of TNFR family members. None of the full-length cDNA clones isolated encoded transmembrane and cytoplasmic domains that would be expected for membrane-bound receptors, suggesting that these cDNAs encode soluble, secreted proteins rather than cell surface receptors. A portion of the human gene spanning nucleotides 1200-1353 shown in Figure 9D was deposited in the Genebank database on November 22, 1995 under accession no. 17188769.

The tissue distribution of the rat and human mRNA was determined as described in Example 2. In rat, mRNA expression was detected in kidney, liver, placenta and heart with the highest expression in the kidney. Expression in skeletal muscle and pancreas was also detected. In humans, expression was detected in the same tissues along with lymph node, thymus, spleen and appendix.

The rat cDNA was expressed in transgenic mice (Example 3) using the liver-specific ApoE promoter expression system. Analysis of expressors showed a marked increase in bone density, particularly in long bones (femurs), vertebrae and flat bones (pelvis). Histological analysis of stained sections of bone showed severe osteopetrosis (see Example 4) indicating a marked imbalance between bone formation and resorption which has led to a marked accumulation of bone and cartilage. A decrease in the number of trabecular osteoclasts in the bones of OPG expressor animals indicate that a significant portion of the activity of the TNFR-related protein may be to prevent bone resorption, a process mediated by osteoclasts. In view of the activity in transgenic expressors, the TNFR-related proteins described herein are termed OPGs.

Using the rat cDNA sequence, mouse and human cDNA clones were isolated (Example 5). Expression of mouse OPG in 293 cells and human OPG in E. coli is described in Examples 7 and 8. Mouse OPG was produced as an Fc fusion which was purified by Protein A affinity chromatography. Also described in Example 7 is the expression of full-length and truncated human and mouse OPG polypeptides in CHO and 293 cells either as fusion polypeptides to the Fc region of human IgG1 or as unfused polypeptides. The expression of full-length and truncated human and mouse OPGs in E. coli either as Fc fusion polypeptides or as unfused polypeptides is described in Example 8. Purification of recombinantly produced mammalian and bacterial OPG is described in Example 10.

The biological activity of OPG was determined using an in vitro osteoclast maturation assay, an in vivo model of interleukin-1 (IL-1) induced

hypercalcemia, and injection studies of bone density in normal mice (see Example 11). The following OPG recombinant proteins produced in CHO or 293 cells demonstrated activity in the in E. coli osteoclast maturation assay: muOPG [22-185]-Fc, muOPG [22-194]-Fc, muOPG [22-401]Fc, muOPG [22-401], huOPG [22-201]-Fc, huOPG [22-401]-Fc. muOPG [22-180]-Fc produced in CHO cells and huOPG met[32-401] produced in E. coli did not demonstrate activity in the in vitro assay.

OPG from several sources was produced as a dimer and to some extent as a higher multimer. Rat OPG [22-401] produced in transgenic mice, muOPG [22-401] and huOPG [22-401] produced as a recombinant polypeptide in CHO cells, and OPG expressed as a naturally occurring product from a cytotoxic T cell line were predominantly dimers and trimers when analyzed on nonreducing SDS gels (see Example 9). Truncated OPG polypeptides having deletions in the region of amino acids 186-401 (e.g., OPG [1-185] and OPG [1-194]) were predominantly monomeric suggesting that the region 186-401 may be involved in self-association of OPG polypeptides. However, huOPG met[32-401] produced in E. coli was largely monomeric.

OPG may be important in regulating bone resorption. The protein appears to act as a soluble receptor of the TNF family and may prevent a receptor-ligand interaction involved in the osteolytic pathway. One aspect of the regulation appears to be a reduction in the number of osteoclasts.

Nucleic Acids

The invention provides for an isolated nucleic acid encoding a polypeptide having at least one of the biological activities of OPG. As described herein, the

biological activities of OPG include, but are not limited to, any activity involving bone metabolism and in particular, include increasing bone density. The nucleic acids of the invention are selected from the following:

a) the nucleic acid sequences as shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124) or complementary strands thereof;

b) the nucleic acids which hybridize under stringent conditions with the polypeptide-encoding region in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124); and

c) nucleic acids which hybridize under stringent conditions with nucleotides 148 through 337 inclusive as shown in Figure 1A.

d) the nucleic acid sequences which are degenerate to the sequences in (a) and (b).

The invention provides for nucleic acids which encode rat, mouse and human OPG as well as nucleic acid sequences hybridizing thereto which encode a polypeptide having at least one of the biological activities of OPG. Also provided for are nucleic acids which hybridize to a rat OPG EST encompassing nucleotides 148-337 as shown in Figure 1A. The conditions for hybridization are generally of high stringency such as 5xSSC, 50% formamide and 42°C described in Example 1 of the specification. Equivalent stringency to these conditions may be readily obtained by adjusting salt and organic solvent concentrations and temperature. The nucleic acids in (b) encompass sequences encoding OPG-related polypeptides which do not undergo detectable hybridization with other known members of the TNF receptor superfamily. In a preferred embodiment, the nucleic acids are as shown in Figures 2B-2C (SEQ ID

NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124).

The length of hybridizing nucleic acids of the invention may be variable since hybridization may occur in part or all of the polypeptide-encoding regions as shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124), and may also occur in adjacent noncoding regions. Therefore, hybridizing nucleic acids may be truncations or extensions of the sequences shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124). Truncated or extended nucleic acids are encompassed by the invention provided they retain one or more of the biological properties of OPG. The hybridizing nucleic acids may also include adjacent noncoding regions which are 5' and/or 3' to the OPG coding region. The noncoding regions include regulatory regions involved in OPG expression, such as promoters, enhance, translational initiation sites, transcription termination sites and the like.

Hybridization conditions for nucleic acids are described in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)

DNA encoding rat OPG was provided in plasmid pMO-B1.1 deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under ATCC accession no. 69970. DNA encoding mouse OPG was provided in plasmid pRcCMV-murine OPG deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under accession no. 69971. DNA encoding human OPG was provided in plasmid pRcCMV - human OPG deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under

accession no. 69969. The nucleic acids of the invention will hybridize under stringent conditions to the DNA inserts of ATCC accession nos. 69969, 69970, and 69971 and have at least one of the biological activities of OPG.

Also provided by the invention are derivatives of the nucleic acid sequences as shown in Figures 2B, 9A and 9B. As used herein, derivatives include nucleic acid sequences having addition, substitution, insertion or deletion of one or more residues such that the resulting sequences encode polypeptides having one or more amino acid residues which have been added, deleted, inserted or substituted and the resulting polypeptide has the activity of OPG. The nucleic acid derivatives may be naturally occurring, such as by splice variation or polymorphism, or may be constructed using site-directed mutagenesis techniques available to the skilled worker. One example of a naturally occurring variant of OPG is a nucleic acid encoding a lys to asn change at residue 3 within the leader sequence (see Example 5). It is anticipated that nucleic acid derivatives will encode amino acid changes in regions of the molecule which are least likely to disrupt biological activity. Other derivatives include a nucleic acid encoding a membrane-bound form of OPG having an extracellular domain as shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124) along with transmembrane and cytoplasmic domains.

In one embodiment, derivatives of OPG include nucleic acids encoding truncated forms of OPG having one or more amino acids deleted from the carboxy terminus. Nucleic acids encoding OPG may have from 1 to 216 amino acids deleted from the carboxy terminus. Optionally, an antibody Fc region may extend from the new carboxy

terminus to yield a biologically active OPG-Fc fusion polypeptide. (see Example 11). In preferred embodiments, nucleic acids encode OPG having the amino acid sequence from residues 22-185, 22-189, 22-194 or 22-201 (using numbering in Figure 9E-F) and optionally, encoding an Fc region of human IgG.

Also included are nucleic acids encoding truncated forms of OPG having one or more amino acids deleted from the amino terminus. Truncated forms include those lacking part or all the 21 amino acids comprising the leader sequence. Additionally, the invention provides for nucleic acids encoding OPG having from 1 to 10 amino acids deleted from the mature amino terminus (at residue 22) and ,optionally, having from 1 to 216 amino acids deleted from the carboxy terminus (at residue 401). Optionally, the nucleic acids may encode a methionine residue at the amino terminus. Examples of such OPG truncated polypeptides are described in Example 8.

Examples of the nucleic acids of the invention include cDNA, genomic DNA, synthetic DNA and RNA. cDNA is obtained from libraries prepared from mRNA isolated from various tissues expressing OPG. In humans, tissue sources for OPG include kidney, liver, placenta and heart. Genomic DNA encoding OPG is obtained from genomic libraries which are commercially available from a variety of species. Synthetic DNA is obtained by chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the fragments to reconstitute part or all of the coding region and flanking sequences (see U.S. Patent No. 4,695,623 describing the chemical synthesis of interferon genes). RNA is obtained most easily by procaryotic expression

vectors which direct high-level synthesis of mRNA, such as vectors using T7 promoters and RNA polymerase.

Nucleic acid sequences of the invention are used for the detection of OPG sequences in biological samples in order to determine which cells and tissues are expressing OPG mRNA. The sequences may also be used to screen cDNA and genomic libraries for sequences related to OPG. Such screening is well within the capabilities of one skilled in the art using appropriate hybridization conditions to detect homologus sequences. The nucleic acids are also useful for modulating the expression of OPG levels by anti-sense therapy or gene therapy. The nucleic acids are also used for the development of transgenic animals which may be used for the production of the polypeptide and for the study of biological activity (see Example 3).

Vectors and Host Cells

Expression vectors containing nucleic acid sequences encoding OPG, host cells transformed with said vectors and methods for the production of OPG are also provided by the invention. An overview of expression of recombinant proteins is found in Methods of Enzymology v. 185, Goeddel, D.V. ed. Academic Press (1990).

Host cells for the production of OPG include procaryotic host cells, such as *E. coli*, yeast, plant, insect and mammalian host cells. *E. coli* strains such as HB101 or JM101 are suitable for expression. Preferred mammalian host cells include COS, CHOd-, 293, CV-1, 3T3, baby hamster kidney (BHK) cells and others. Mammalian host cells are preferred when post-translational modifications, such as glycosylation and polypeptide processing, are important for OPG activity. Mammalian expression allows for the production of

secreted polypeptides which may be recovered from the growth medium.

Vectors for the expression of OPG contain at a minimum sequences required for vector propogation and for expression of the cloned insert. These sequences include a replication origin, selection marker, promoter, ribosome binding site, enhancer sequences, RNA splice sites and transcription termination site. Vectors suitable for expression in the aforementioned host cells are readily available and the nucleic acids of the invention are inserted into the vectors using standard recombinant DNA techniques. Vectors for tissue-specific expression of OPG are also included. Such vectors include promoters which function specifically in liver, kidney or other organs for production in mice, and viral vectors for the expression of OPG in targeted human cells.

Using an appropriate host-vector system, OPG is produced recombinantly by culturing a host cell transformed with an expression vector containing nucleic acid sequences encoding OPG under conditions such that OPG is produced, and isolating the product of expression. OPG is produced in the supernatant of transfected mammalian cells or in inclusion bodies of transformed bacterial host cells. OPG so produced may be purified by procedures known to one skilled in the art as described below. The expression of OPG in mammalian and bacterial host systems is described in Examples 7 and 8. Expression vectors for mammalian hosts are exemplified by plasmids such as pDSRα described in PCT Application No. 90/14363. Expression vectors for bacterial host cells are exemplified by plasmids pAMG21 and pAMG22-His described in Example 8. Plasmid pAMG21 was deposited with the American Type

Culture Collection, Rockville, MD on July 24, 1996 under accession no. 98113. Plasmid pAMG22-His was deposited with the American Type Culture Collection, Rockville, MD on July 24, 1996 under accession no. 98112. It is anticipated that the specific plasmids and host cells described are for illustrative purposes and that other available plasmids and host cells could also be used to express the polypeptides.

The invention also provides for expression of OPG from endogenous nucleic acids by *in vivo* or *ex vivo* recombination events to allow modulation of OPG from the host chromosome. Expression of OPG by the introduction of exogenous regulatory sequences (e.g. promoters or enhancers) capable of directing the production of OPG from endogenous OPG coding regions is also encompassed. Stimulation of endogenous regulatory sequences capable of directing OPG production (e.g. by exposure to transcriptional enhancing factors) is also provided by the invention.

Polypeptides

The invention provides for OPG, a novel member of the TNF receptor superfamily, having an activity associated with bone metabolism and in particular having the activity of inhibiting bone resorption thereby increasing bone density. OPG refers to a polypeptide having an amino acid sequence of mouse, rat or human OPG or a derivative thereof having at least one of the biological activities of OPG. The amino acid sequences of rat, mouse and human OPG are shown in Figures 2B-2C (SEQ ID NO:121), 9A-9B (SEQ ID NO:123), and 9C-9D (SEQ ID NO:125) respectively. A derivative of OPG refers to a polypeptide having an addition, deletion, insertion or substitution of one or more amino acids such that the

resulting polypeptide has at least one of the biological activities of OPG. The biological activities of OPG include, but are not limited to, activities involving bone metabolism. Preferably, the polypeptides will have the amino terminal leader sequence of 21 amino acids removed.

OPG polypeptides encompassed by the invention include rat [1-401], rat [22-180], rat [22-401], rat [22-401]-Fc fusion, rat [1-180]-Fc fusion, mouse [1-401], mouse [1-180], mouse [22-401], human [1-401], mouse [22-180], human [22-401], human [22-180], human [1-180], human [22-180]-Fc fusion and human met-32-401. Amino acid numbering is as shown in SEQ ID NO:121 (rat), SEQ ID NO:123 (mouse) and SEQ ID NO:125 (human). Also encompassed are polypeptide derivatives having deletions or carboxy-terminal truncations of part or all of amino acids residues 180-401 of OPG; one or more amino acid changes in residues 180-401; deletion of part or all of a cysteine-rich domain of OPG, in particular deletion of the distal (carboxy-terminal) cysteine-rich domain; and one or more amino acid changes in a cysteine-rich domain, in particular in the distal (carboxy-terminal) cysteine-rich domain. In one embodiment, OPG has from 1 to about 216 amino acids deleted from the carboxy terminus. In another embodiment, OPG has from 1 to about 10 amino acids deleted from the mature amino terminus (wherein the mature amino terminus is at residue 22) and, optionally, has from 1 to about 216 amino acids deleted from the carboxy terminus.

Additional OPG polypeptides encompassed by the invention include the following: human [22-180]-Fc fusion, human [22-201]-Fc fusion, human [22-401]-Fc fusion, mouse [22-185]-Fc fusion, mouse [22-194]-Fc fusion. These polypeptides are produced in mammalian

host cells, such as CHO or 293 cells, Additional OPG polypeptides encompassed by the invention which are expressed in procaryotic host cells include the following: human met[22-401], Fc-human met[22-401] fusion (Fc region is fused at the amino terminus of the full-length OPG coding sequence as described in Example 8), human met[22-401]-Fc fusion (Fc region fused to the full-lengh OPG sequence), Fc-mouse met[22-401] fusion, mouse met[22-401]-Fc fusion, human met[27-401], human met[22-185], human met[22-189], human met[22-194], human met[22-194] (P25A), human met [22-194] (P26A), human met[27-185], human met[27-189], human met[27-194], human met-arg-gly-ser-(his)$_6$ [22-401], human met-lys [22-401], human met-(lys)$_3$-[22-401], human met[22-401]-Fc (P25A), human met[22-401](P25A), human met[22-401](P26A), human met[22-401] (P26D), mouse met[22-401], mouse met[27-401], mouse met[32-401], mouse met[27-180], mouse met[22-189], mouse met[22-194], mouse met[27-189], mouse met[27-194], mouse met-lys[22-401], mouse HEK[22-401](A45T), mouse met-lys-(his)$_7$[22-401], mouse met-lys[22-401]-(his)$_7$ and mouse met[27-401] (P33E, G36S, A45P). It is understood that the above OPG polypeptides produced in procaryotic host cells have an amino-terminal methionine residue, if such a residue is not indicated. In specific examples, OPG-Fc fusion were produced using a 227 amino acid region of human IgG1-γ1 was used having the sequence as shown in Ellison et al. (Nuc. Acids Res. 10, 4071-4079 (1982)). However, variants of the Fc region of human IgG may also be used.

Analysis of the biological activity of carboxy-terminal OPG truncations fused to the human IgG1 Fc region indicates a portion of OPG of about 164 amino acids which is required for activity. This region encompasses amino acids 22-185, preferably those in

Figure 9C-9D (SEQ ID NO:125), and comprises four cysteine-rich domains characteristic of the cysteine-rich domains of TNFR extraceullular domains.

Using the homology between OPG and the extracellular ligand binding domains of TNF receptor family members, a three-dimensional model of OPG was generated based upon the known crystal structure of the extracellular domain of TNFR-I (see Example 6). This model was used to identify those residues within OPG which may be important for biological activity. Cysteine residues that are involved in maintaining the structure of the four cysteine-rich domains were identified. The following disulfide bonds were identified in the model: Domain 1: cys41 to cys54, cys44 to cys62, tyr23 and his 66 may act to stabilize the structure of this domain; Domain 2: cys65 to cys80, cys83 to cys98, cys87 to cys105; Domain 3: cys107 to cys118, cys124 to cys142; Domain 4: cys145 to cys160, cys166 to cys185. Residues were also identified which were in close proximity to TNFβ as shown in Figures 11 and 12A-12B. In this model, it is assumed that OPG binds to a corresponding ligand; TNFβ was used as a model ligand to simulate the interaction of OPG with its ligand. Based upon this modeling, the following residues in OPG may be important for ligand binding: glu34, lys43, pro66 to gln91 (in particular, pro66, his68, tyr69, tyr70, thr71, asp72, ser73, his76, ser77, asp78, glu79, leu81, tyr82, pro85, val86, lys88, glu90 and gln91), glu153 and ser155.

Alterations in these amino acid residues, either singly or in combination, may alter the biological activity of OPG. For example, changes in specific cysteine residues may alter the structure of individual cysteine-rich domains, whereas changes in

residues important for ligand binding may affect physical interactions of OPG with ligand. Structural models can aid in identifying analogs which have more desirable properties, such as enhanced biological activity, greater stability, or greater ease of formulation.

The invention also provides for an OPG multimer comprising OPG monomers. OPG appears to be active as a multimer (e.g, dimer, trimer of a higher number of monomers). Preferably, OPG multimers are dimers or trimers. OPG multimers may comprise monomers having the amino acid sequence of OPG sufficient to promote multimer formation or may comprise monomers having heterologous sequences such as an antibody Fc region. Analysis of carboxy-terminal deletions of OPG suggest that at least a portion of the region 186-401 is involved in association of OPG polypeptides. Substitution of part or all of the region of OPG amino acids 186-401 with an amino acid sequence capable of self-association is also encompassed by the invention. Alternatively, OPG polypeptides or derivatives thereof may be modified to form dimers or multimers by site directed mutagenesis to create unpaired cysteine residues for interchain disulfide bond romation, by photochemical crosslinking, such as exposure to ultraviolet light, or by chemical crosslinking with bifunctional linker molecules such as bifunctional polyethylene glycol and the like.

Modifications of OPG polypeptides are encompassed by the invention and include post-translational modifications (e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked

or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Further modifications of OPG include chimeric proteins wherein OPG is fused to a heterologous amino acid sequence. The heterologous sequence may be any sequence which allows the resulting fusion protein to retain the activity of OPG. The heterologous sequences include for example, immunoglobulin fusions, such as Fc fusions, which may aid in purification of the protein. A heterologous sequence which promotes association of OPG monomers to form dimers, trimers and other higher multimeric forms is preferred.

The polypeptides of the invention are isolated and purified from other polypeptides present in tissues, cell lines and transformed host cells expressing OPG, or purified from components in cell cultures containing the secreted protein. In one embodiment, the polypeptide is free from association with other human proteins, such as the expression product of a bacterial host cell.

Also provided by the invention are chemically modified derivatives of OPG which may provide additional advantages such as increasing stability and circulating time of the polypeptide, or decreasing immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined

positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1kDa and about 100kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g. EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20: 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and th C-terminal amino acid residue. Sulfhydrl groups may also be used as a reactive group

for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire N-terminally chemically modified protein. Using polyethylene glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemically modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

Synthetic OPG dimers may be prepared by various chemical crosslinking procedures. OPG monomers may be chemically linked in any fashion that retains or enhances the biological activity of OPG. A variety of chemical crosslinkers may be used depending upon which properties of the protein dimer are desired. For example, crosslinkers may be short and relatively rigid or longer and more flexible, may be biologically

reversible, and may provide reduced immunogenicity or longer pharmacokinetic half-life.

In one example, OPG molecules are linked through the amino terminus by a two step synthesis (see Example 12). In the first step, OPG is chemically modified at the amino terminus to introduce a protected thiol, which after purification is deprotected and used as a point of attachment for site-specific conjugation through a variety of crosslinkers with a second OPG molecule. Amino-terminal crosslinks include, but are not limited to, a disulfide bond, thioether linkages using short-chain, bis-functional aliphatic crosslinkers, and thioether linkages to variable length, bifunctional polyethylene glycol crosslinkers (PEG "dumbbells"). Also encompassed by PEG dumbbell synthesis of OPG dimers is a byproduct of such synthesis, termed a "monobell". An OPG monobell consists of a monomer coupled to a linear bifunctional PEG with a free polymer terminus. Alternatively, OPG may be crosslinked directly through a variety of amine specific homobifunctional crosslinking techniques which include reagents such as: diethylenetriaminepentaacetic dianhydride (DTPA), p-benzoquinone (pBQ) or bis(sulfosuccinimidyl) suberate (BS^3) as well as others known in the art. It is also possible to thiolate OPG directly with reagents such as iminothiolane in the presence of a variety of bifunctional, thiol specific crosslinkers, such as PEG bismaleimide, and achieve dimerization and/or dumbbells in a one step process.

A method for the purification of OPG from natural sources and from transfected host cells is also included. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purifi d protein. The

chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-OPG antibody or biotin-streptavidin affinity complex and the like.

Antibodies

Also encompassed by the invention are antibodies specifically binding to OPG. Antigens for the generation of antibodies may be full-length polypeptides or peptides spanning a portion of the OPG sequence. Immunological procedures for the generation of polyclonal or monoclonal antibodies reactive with OPG are known to one skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y. (1988)). Antibodies so produced are characterized for binding specificity and epitope recognition using standard enzyme-linked immunosorbent assays. Antibodies also include chimeric antibodies having variable and constant domain regions derived from different species. In one embodiment, the chimeric antibodies are humanized antibodies having murine variable domains and human constant domains. Also encompassed are complementary determining regions grafted to a human framework (so-called CDR-grafted antibodies). Chimeric and CDR-grafted antibodies are made by recombinant methods known to one skilled in the art. Also encompassed are human antibodies made in mice.

Anti-OPG antibodies of the invention may be used as an affinity reagent to purify OPG from biological samples (see Example 10). In one method, the antibody is immobilized on CnBr-activated Sepharose and a column of antibody-Sepharose conjugate is used to

remove OPG from liquid samples. Antibodies are also used as diagnostic reagents to detect and quantitate OPG in biological samples by methods described below.

Pharmaceutical compositions

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the polypeptide of the invention together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The term "therapeutically effective amount" means an amount which provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH values and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascrobic acid or sodium metabisulfite. Also encompassed are compositions comprising OPG modified with water soluble polymers to increase solubility or stability. Compositions may also comprise incorporation of OPG into liposomes, microemulsions, micelles or vesicles for controlled delivery over an extended period of time. Specifically, OPG compositions may comprise incorporation into polymer matricies such as hydrogels, silicones, polyethylenes, ethylene-vinyl acetate copolymers, or biodegradable polymers. Examples of hydrogels include polyhydroxyalkylmethacrylates (p-HEMA), polyacrylamide, polymethacrylamide, polyvinylpyrrolidone, polyvinyl alcohol and various polyelectrolyte complexes. Examples of biodegradable polymers include polylactic acid (PLA), polyglycolic

acid (PGA), copolymers of PLA and PGA, polyamides and copolymers of polyamides and polyesters. Other controlled release formulations include microcapsules, microspheres, macromolecular complexes and polymeric beads which may be administered by injection.

Selection of a particular composition will depend upon a number of factors, including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of component suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

Compositions of the invention may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be suitable for the delivery of part or all of the OPG coding region to cells and tissues as part of an anti-sense or gene therapy regimen.

Methods of Treatment

Bone tissue provides support for the body and consists of mineral (largely calcium and phosphorous), a matrix of collagenous and noncollagenous proteins, and c lls. Three types of cells found in bone, osteocytes,

osteoblasts and osteoclasts, are involved in the dynamic process by which bone is continually formed and resorbed. Osteoblasts promote formation of bone tissue whereas osteoclasts are associated with resorption. Resorption, or the dissolution of bone matrix and mineral, is a fast and efficient process compared to bone formation and can release large amounts of mineral from bone. Osteoclasts are involved in the regulation of the normal remodeling of skeletal tissue and in resorption induced by hormones. For instance, resorption is stimulated by the secretion of parathyroid hormone in response to decreasing concentrations of calcium ion in extracellular fluids. In contrast, inhibition of resorption is the principal function of calcitonin. In addition, metabolites of vitamin D alter the responsiveness of bone to parathyroid hormone and calcitonin.

After skeletal maturity, the amount of bone in the skeleton reflects the balance (or imbalance) of bone formation and bone resorption. Peak bone mass occurs after skeletal maturity prior to the fourth decade. Between the fourth and fifth decades, the equilibrium shifts and bone resorption dominates. The inevitable decrease in bone mass with advancing years starts earlier in females than males and is distinctly accelerated after menopause in some females (principally those of Caucasian and Asian descent).

Osteopenia is a condition relating generally to any decrease in bone mass to below normal levels. Such a condition may arise from a decrease in the rate of bone synthesis or an increase in the rate of bone destruction or both. The most common form of osteopenia is primary osteoporosis, also referred to as postmenopausal and senile osteoporosis. This form of

osteoporosis is a consequence of the universal loss of bone with age and is usually a result of increase in bone resorption with a normal rate of bone formation. About 25 to 30 percent of all white females in the United States develop symptomatic osteoporosis. A direct relationship exists between osteoporosis and the incidence of hip, femoral, neck and inter-trochanteric fracture in women 45 years and older. Elderly males develop symptomatic osteoporosis between the ages of 50 and 70, but the disease primarily affects females.

The cause of postmenopausal and senile osteoporosis is unknown. Several factors have been identified which may contribute to the condition. They include alteration in hormone levels accompanying aging and inadequate calcium consumption attributed to decreased intestinal absorption of calcium and other minerals. Treatments have usually included hormone therapy or dietary supplements in an attempt to retard the process. To date, however, an effective treatment for bone loss does not exist.

The invention provides for a method of treating a bone disorder using a therapeutically effective amount of OPG. The bone disorder may be any disorder characterized by a net bone loss (osteopenia or osteolysis). In general, treatment with OPG is anticipated when it is necessary to suppress the rate of bone resorption. Thus treatment may be done to reduce the rate of bone resorption where the resorption rate is above normal or to reduce bone resorption to below normal levels in order to compensate for below normal levels of bone formation.

Conditions which are treatable with OPG include the following:

Osteoporosis, such as primary osteoporosis, endocrine osteoporosis (hyperthyroidism, hyperparathryoidism, Cushing's syndrome, and acromegaly), hereditary and congenital forms of osteoporosis (osteogenesis imperfecta, homocystinuria, Menkes' syndrome, and Riley-Day syndrome) and osteoporosis due to immobilization of extremities.

Paget's disease of bone (osteitis deformans) in adults and juveniles

Osteomyelitis, or an infectious lesion in bone, leading to bone loss.

Hypercalcemia resulting from solid tumors (breast, lung and kidney) and hematologic malignacies (multiple myeloma, lymphoma and leukemia), idiopathic hypercalcemia, and hypercalcemia associated with hyperthryoidism and renal function disorders.

Osteopenia following surgery, induced by steroid administration, and associated with disorders of the small and large intestine and with chronic hepatic and renal diseases.

Osteonecrosis, or bone cell death, associated with traumatic injury or nontraumatic necrosis associated with Gaucher's disease, sickle cell anemia, systemic lupus erythematosus and other conditions.

Bone loss due to rheumatoid arthritis.

Periodontal bone loss.

Osteolytic metastasis

It is understood that OPG may be used alone or in conjunction with other factors for the treatment of bone disorders. In one embodiment, osteoprotegerein is used in conjunction with a therapeutically effective amount of a factor which stimulates bone formation. Such factors include but are not limited to the bone morphogenic factors designated BMP-1 through BMP-12,

transforming growth factor-β (TGF-β) and TGF-β family members, interleukin-1 inhibitors, TNFα inhibitors, parathyroid hormone and analogs thereof, parathyroid related protein and analogs thereof, E series prostaglandins, bisphosphonates (such as alendronate and others), and bone-enhancing minerals such as fluoride and calcium.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Identification and isolation of the rat OPG cDNA

Materials and methods for cDNA cloning and analysis are described in Maniatis et al, ibid. Polymerase chain reactions (PCR) were performed using a Perkin-Elmer 9600 thermocycler using PCR reaction mixture (Boehringer-Mannheim) and primer concentrations specified by the manufacturer. In general, 25-50 μl reactions were denatured at 94°C, followed by 20-40 cycles of 94°C for 5 seconds, 50-60°C for 5 seconds, and 72°C for 3-5 minutes. Reactions were the treated for 72 °C for 3-5 minutes. Reactions were then analyzed by gel electrophoresis as described in Maniatis et al., ibid.

A cDNA library was constructed using mRNA isolated from embryonic d20 intestine for EST analysis (Adams et al. Science 252, 1651-1656 (1991)). Rat embryos were dissected, and the entire developing small and large intestine removed and washed in PBS. Total

cell RNA was purified by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi Anal. Biochem. 162, 156-159, (1987)). The poly (A+) mRNA fraction was obtained from the total RNA preparation by adsorption to, and elution from, Dynabeads Oligo (dT)25 (Dynal Corp) using the manufacturer's recommended procedures. A random primed cDNA library was prepared using the Superscript Plasmid System (Gibco BRL, Gaithersburg, Md). The random cDNA primer containing an internal Not I restriction site was used to initiate first strand synthesis and had the following sequence:

5'-AAAGGAAGGAAAAAAGCGGCCGCTACANNNNNNNNT-3' (SEQ ID NO:1)
Not I

For the first strand synthesis three separate reactions were assembled that contained 2.5 µg of poly(A) RNA and 120 ng, 360 ng or 1,080 ng of random primer. After second strand synthesis, the reaction products were separately extracted with a mixture of phenol:choroform:isoamyl alcohol (25:24:1 ratio), and then ethanol precipitated. The double strand (ds) cDNA products of the three reactions were combined and ligated to the following ds oligonucleotide adapter:

5'-TCGACCCACGCGTCCG-3' (SEQ ID NO:2)
3'-GGGTGCGCAGGCp-5' (SEQ ID NO:3)

After ligation the cDNA was digested to completion with Not I, extracted with phenol:chloroform:isoamyl (25:24:1) alcohol and ethanol precipitated. The resuspended cDNA was then size fractionated by gel filtration using premade columns provided with the Superscript Plasmid System (Gibco BRL, Gaithersburg, Md) as recommended by the manufacturer.

The two fractions containing the largest cDNA products were pooled, ethanol precipitated and then directionally ligated into Not I and Sal I digested pMOB vector DNA (Strathmann et al, 1991). The ligated cDNA was introduced into competent ElectroMAX DH10B E. coli (Gibco BRL, Gaithersburg, MD) by electroporation. For automated sequence analysis approximately 10,000 transformants were plated on 20cm x 20cm agar plates containing ampicillin supplemented LB nutrient media. The colonies that arose were picked and arrayed onto 96 well microtiter plates containing 200 ml of L-broth, 7.5% glycerol, and 50 µg/ml ampicillin. The cultures were grown overnight at 37°C, a duplicate set of microtiter plates were made using a sterile 96 pin replicating tool, then both sets were stored at -80°C for further analysis. For full-length cDNA cloning approximately one million transformants were plated on 96 bacterial ampicillin plates containing about 10,000 clones each. The plasmid DNA from each pool was separately isolated using the Qiagen Plasmid Maxi Kit (Qiagen Corp.,Germany) and arrayed into 96 microtiter plates for PCR analyses.

To sequence random fetal rat intestine cDNA clones, glycerol stocks were thawed, and small aliquots diluted 1:25 in distilled. Approximately 3.0 ul of diluted bacterial cultures were added to PCR reaction mixture (Boehringer-Mannheim) containing the following oligonucleotides:

5'-TGTAAAACGACGGCCAGT-3' (SEQ ID NO:4)
5'-CAGGAAACAGCTATGACC-3' (SEQ ID NO:5)

The reactions wer incubated in a thermocycler (Perkin-Elmer 9600) with the following cycle conditions:

94 C for 2 minutes; 30 cycles of 94°C for 5 seconds, 50°C for 5 seconds, and 72°C for 3 minutes.; 72°C for 4 minutes. After incubation in the thermocycler, the reactions were diluted with 2.0 mL of water. The amplified DNA fragments were further purified using Centricon columns (Princeton Separations) using the manufacturer's recommended procedures. The PCR reaction products were sequenced on an Applied Biosystems 373A automated DNA sequencer using T3 primer (oligonucleotide 353-23; 5'-CAATTAACCCTCACTAAAGG-3') (SEQ ID NO:6) Taq dye-terminator reactions (Applied Biosystems) following the manufacturer's recommended procedures.

The resulting 5' nucleotide sequence obtained from randomly picked cDNA clones translated and then compared to the existing database of known protein sequences using a modified version of the FASTA program (Pearson et al. Meth. Enzymol. 183, (1990)). Translated sequences were also analysed for the presence of a specific cysteine-rich protein motif found in all known members of the tumor necrosis factor receptor (TNFR) superfamily (Smith et al. Cell 76, 959-962 (1994)), using the sequence profile method of Gribskov et al. (Proc. Natl. Acad. Sci. USA 83, 4355-4359 (1987)), as modified by Luethy et al. (Protein Science 3, 139-146 (1994)).

Using the FASTA and Profile search data, an EST, FRI-1 (Fetal Rat Intestine-1), was identified as a possible new member of the TNFR superfamily. FRI-1 contained an approximately 600 bp insert with a LORF of about 150 amino acids. The closest match in the database was the human type II TNFR (TNFR-2). The region compared showed an ~43% homology between TNFR-2 and FRI-1 over this 150 aa LORF. Profile analysis using the first and second cysteine-rich repeats of the TNFR

superfamily yielded a Z score of ~8, indicating that the FRI-1 gene possibly encodes a new family member. To deduce the structure of the FRI-1 product, the fetal rat intestine cDNA library was screened for full length clones. The following oligonucleotides were derived from the original FRI-1 sequence:

5'-GCATTATGACCCAGAAACCGGAC-3' (SEQ ID NO:7)
5'-AGGTAGCGCCCTTCCTCACATTC-3' (SEQ ID NO:8)

These primers were used in PCR reactions to screen 96 pools of plasmid DNA, each pool containing plasmid DNA from 10,000 independent cDNA clones. Approximately 1 ug of plasmid pool DNA was amplified in a PCR reaction mixture (Boehringer-Mannheim) using a Perkin-Elmer 96 well thermal cycler with the following cycle conditions: 2 min at 94°C, 1 cycle; 15 sec at 94°C, then 45 sec at 65°C, 30 cycles; 7 min at 65°C, 1 cycle. PCR reaction products were analysed by gel electrophoresis. 13 out of 96 plasmid DNA pools gave rise to amplified DNA products with the expected relative molecular mass.

DNA from one positive pool was used to transform competent ElectroMAX DH10B E. coli (Gibco BRL, Gaithersburg, MD) as described above. Approximately 40,000 transformants were plated onto sterile nitrocellulose filters (BA-85, Schleicher and Schuell), and then screened by colony hybridization using a ^{32}P-dCTP labelled version of the PCR product obtained above. Filters were prehybridized in 5X SSC, 50% deionized formamide, 5X Denhardt's solution, 0.5% SDS, and 100 ug/ml denatured salmon sperm DNA for 2-4 hours at 42°C. Filters were then hybridized in 5X SSC, 50% deionized formamide, 2X Denhardt's solution, 0.1% SDS, 100 μg/ml

denatured salmon sperm DNA, and ~5 ng/ml of labelled probe for ~18 hours at 42°C. The filters were then washed in 2X SSC for 10 min at RT, 1X SSC for 10 min at 55°C, and finally in 0.5X SSC for 10-15 min at 55°C. Hybridizing clones were detected following autoradiography, and then replated onto nitrocellulose filters for secondary screening. Upon secondary screening, a plasmid clone (pB1.1) was isolated, then amplified in L-broth media containing 100 ug/ml ampicillin and the plasmid DNA obtained. Both strands of the 2.4 kb pB1.1 insert were sequenced.

The pB1.1 insert sequence was used for a FASTA search of the public database to detect any existing sequence matches and/or similarities. No matches to any known genes or EST's were found, although there was an approximate 45% similarity to the human and mouse TNFR-2 genes. A methionine start codon is found at bp 124 of the nucleotide sequence, followed by a LORF encoding 401 aa residues that terminates at bp 1327. The 401 aa residue product is predicted to have a hydrophobic signal peptide of approximately 31 residues at its N-terminus, and 4 potential sites of N-linked glycosylation. No hydrophobic transmembrane spanning sequence was identified using the PepPlot program (Wisconsin GCG package, version 8.1). The deduced 401 aa sequence was then used to search the protein database. Again, there were no existing matches, although there appeared to be a strong similarity to many members of the TNFR superfamily, most notably the human and mouse TNFR-2. A sequence alignment of this novel protein with known members of the TNFR-superfamily was prepared using the Pileup program, and then modified by PrettyPlot (Wisconsin GCG package, version 8.1). This alignment shows a clear homology between the full

length FRI-1 gene product and all other TNFR family members. The homologus region maps to the extracellular domain of TNFR family members, and corresponds to the three or four cysteine-rich repeats found in the ligand binding domain of these proteins. This suggested that the FRI-1 gene encoded a novel TNFR family member. Since no transmembrane spanning region was detected we predicted that this may be a secreted receptor, similar to TNFR-1 derived soluble receptors (Kohno et al. Proc. Natl. Acad. Sci. USA 87, 8331-8335 (1990)). Due to the apparent biological activity of the FRI-1 gene (*vide infra*), the product was named Osteoprotegerin (OPG).

EXAMPLE 2

OPG mRNA Expression Patterns in Tissues

Multiple human tissue northern blots (Clonetech) were probed with a ^{32}P-dCTP labelled FRI-1 PCR product to detect the size of the human transcript and to determine patterns of expression. Northern blots were prehybridized in 5X SSPE, 50% formamide, 5X Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA for 2-4 hr at 42°C. The blots were then hybridized in 5X SSPE, 50% formamide, 2X Denhardt's solution, 0.1% SDS, 100 μg/ml denatured salmon sperm DNA, and 5 ng/ml labelled probe for 18-24 hr at 42°C. The blots were then washed in 2X SSC for 10 min at RT, 1X SSC for 10 min at 50°C, then in 0.5X SSC for 10-15 min.

Using a probe derived from the rat gene, a predominant mRNA species with a relative molecular mass of about 2.4 kb is detected in several tissues, including kidn y, liver, placenta, and heart. Highest

levels are detected in the kidney. A large mRNA species of Mr 4.5 and 7.5 kb was detected in skeletal muscle and pancreas. In human fetal tissue, kidney was found to express relatively high levels of the 2.4 kb mRNA. Using a human probe (vide infra), only the 2.4 kb transcript is detected in these same tissues. In addition, relatively high levels of the 2.4 kb transcript was detected in the lymph node, thymus, spleen and appendix. The size of the transcript detected by both the rat and human Osteosprotegerin gene is almost identical to the length of the rat pB1.1 FRI-1 insert, suggesting it was a full length cDNA clone.

EXAMPLE 3

Systemic delivery of OPG in transgenic mice

The rat OPG clone pB1.1 was used as template to PCR amplify the coding region for subcloning into an ApoE-liver specific expression vector (Simonet et al. J. Clin. Invest. 94, 1310-1319 (1994), and PCT Application No. US94/11675 and co-owned U.S. Serial No. 08/221,767. The following 5' and 3' oligonucleotide primers were used for PCR amplification, respectively:

5'-GACTAGTCCCACAATGAACAAGTGGCTGTG-3' (SEQ ID NO:9)
5'-ATAAGAATGCGGCCGCTAAACTATGAAACAGCCCAGTGACCATTC-3' (SEQ ID NO:10)

The PCR reaction mixture (Boehringer-Mannheim) was treated as follows: 94°C for 1 minute, 1 cycle; 94°C for 20 sec, 62°C for 30 sec, and 74 C for 1 minute, 25 cycles. Following amplification, the samples were purified over Qiagen PCR columns and digested overnight with SpeI and NotI restriction enzymes. The digested

products were extracted and precipitated and subcloned into the ApoE promoter expression vector. Prior to microinjecting the resulting clone, HE-OPG, it was sequenced to ensure it was mutation-free.

The HE-OPG plasmid was purified through two rounds of CsCl density gradient centrifugation. The purified plasmid DNA was digested with XhoI and Ase I, and the 3.6 kb transgene insert was purified by gel electrophoresis. The purified fragment was diluted to a stock injection solution of 1 µg/ml in 5 mM Tris, pH 7.4, 0.2 mM EDTA. Single-cell embryos from BDF1 x BDF1-bred mice were injected essentially as described (Brinster et al., Proc. Natl. Acad. Sci. USA 82, 4338 (1985)), except that injection needles were beveled and siliconized before use. Embryos were cultured overnight in a CO_2 incubator and 15 to 20 2-cell embryos were transferred to the oviducts of pseudopregnant CD1 female mice.

Following term pregnancy, 49 offspring were obtained from implantation of microinjected embryos. The offspring were screened by PCR amplification of the integrated transgene in genomic DNA samples. The target region for amplification was a 369 bp region of the human Apo E intron which was included in the expression vector. The oligos used for PCR amplification were:

5'- GCC TCT AGA AAG AGC TGG GAC-3' (SEQ ID NO:11)
5'- CGC CGT GTT CCA TTT ATG AGC-3' (SEQ ID NO:12)

The conditions for PCR were: 94°C for 2 minute, 1 cycle; 94°C for 1 min, 63°C for 20 sec, and 72°C for 30 sec, 30 cycles. Of the 49 original offspring, 9 were identified as PCR positive transgenic founders.

At 8-10 weeks of age, five transgenic founders (2, 11, 16, 17, and 28) and five controls (1, 12, 15, 18, and 30) were sacrificed for necropsy and pathological analysis. Liver was isolated from the remaining 4 founders by partial hepatectomy. For partial hepatectomy, the mice were anesthetized and a lobe of liver was surgically removed. Total cellular RNA was isolated from livers of all transgenic founders, and 5 negative control littermates as described (McDonald et al. Meth. Enzymol. 152, 219 (1987)). Northern blot analysis was performed on these samples to assess the level of transgene expression. Approximately 10ug of total RNA from each animal liver was resolved by electrophoresis denaturing gels (Ogden et al. Meth. Enzymol 152, 61 (1987)), then transferred to HYBOND-N nylon membrane (Amersham), and probed with ^{32}P dCTP-labelled pB1.1 insert DNA. Hybridization was performed overnight at 42°C in 50% Formamide, 5 x SSPE, 0.5% SDS, 5 x Denhardt's solution, 100 µg/ml denatured salmon sperm DNA and 2-4 x 10^6 cpm of labeled probe/ml of hybridization buffer. Following hybridization, blots were washed twice in 2 x SSC, 0.1% SDS at room temperature for 5 min each, and then twice in 0.1 x SSC, 0.1% SDS at 55°C for 5-10 min each. Expression of the transgene in founder and control littermates was determined following autoradiography.

The northern blot data indicate that 7 of the transgenic founders express detectable levels of the transgene mRNA (animal #'s 2,11,16,17,22,33,and 45). The negative control mice and one of the founders (#28) expressed no transgene-related mRNA. Since OPG is predicted to be a secreted protein, overexpression of transgene mRNA should b a proxy for the level of systemically delivered gene product. Of the PCR and

northern blot positive mice, animal 2, 17 and 22 expressed the highest levels of transgene mRNA, and may show more extensive biological effects on host cells and tissues.

EXAMPLE 4

Biological activity of OPG

Five of the transgenic mice (animals 2,11,16,17 and 28) and 5 control littermates (animals 1,12,15,18, and 30) were sacrificed for necropsy and pathological analysis using the following procedures: Prior to euthanasia, all animals had their identification numbers verified, then were weighed, anesthetized and blood drawn. The blood was saved as both serum and whole blood for a complete serum chemistry and hematology panel. Radiography was performed just after terminal anesthesia by lethal CO2 inhalation, and prior to the gross dissection. Following this, tissues were removed and fixed in 10% buffered Zn-Formalin for histological examination. The tissues collected included the liver, spleen, pancreas, stomach, duodenum, ileum, colon, kidney, reproductive organs, skin and mammary glands, bone, brain, heart, lung, thymus, trachea, eosphagus, thyroid, jejunem, cecum, rectum, adrenals, urinary bladder, and skeletal muscle. Prior to fixation the whole organ weights were determined for the liver, stomach, kidney, adrenals, spleen, and thymus. After fixation the tissues were processed into paraffin blocks, and 3 um sections were obtained. Bone tissue was decalcified using a formic acid solution, and all sections were stained with hematoxylin and eosin. In addition, staining with

Gomori's reticulin and Masson's trichrome were performed on certain tissues. Enzyme histochemistry was performed to determine the expression of tartrate resistant acid phosphatase (TRAP), an enyzme highly expressed by osteoclasts, multinucleated bone-resorbing cells of monocyte-macrophage lineage. Immunohistochemistry for BrdU and F480 monocyte-macrophage surface antigen was also performed to detect replicating cells and cells of the monocyte-macrophage lineage, respectively. To detect F480 surface antigen expression, formalin fixed, paraffin embedded 4μm sections were deparaffinized and hydrated to deionized water. The sections were quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw, Pittsburgh, PA), and incubated in rat monoclonal anti-mouse F480 (Harlan, Indianapolis, IN). This antibody was detected by biotinylated rabbit anti-rat immunoglobulins, peroxidase conjugated strepavidin (BioGenex San Ramon, CA) with DAB as chromagen (BioTek, Santa Barbara, CA). Sections were counterstained with hematoxylin.

Upon gross dissection and observation of visceral tissues, no abnormalities were found in the transgene expressors or control littermates. Analysis of organ weight indicate that spleen size increased by approximately 38% in the transgenic mice relative to controls. There was a slight enlargement of platelet size and increased circulating unstained cells in the transgene expressors. There was a marginal decrease in platelet levels in the transgene expressors. In addition, the serum uric acid, urea nitrogen, and alkaline phosphatase levels all trended lower in the transgene expressors. The expressors were found to have increased radiodensity of the skeleton, including long bones (femurs), vertebrae, and flat bones (pelvis). The

relative size of femurs in the expressors were not different from the the control mice.

Histological analysis of stained sections of bone from the OPG expressors show severe osteopetrosis with the presence of cartilage remnants from the primary spongiosa seen within bone trabeculae in the diaphysis of the femur. A clearly defined cortex was not identifiable in the sections of femur. In normal animals, the central diaphysis is filled with bone marrow. Sections of vertebra also show osteopetrotic changes implying that the OPG-induced skeletal changes were systemic. The residual bone marrow showed predominantly myeloid elements. Megakaryocytes were present. Reticulin stains showed no evidence for reticulin deposition. Immunohistochemistry for F480, a cell surface antigen expressed by cells of monocyte-macrophage derivation in the mouse, showed the presence of F480 positive cells in the marrow spaces. Focally, flattened F480 positive cells could be seen directly adjacent to trabecular bone surfaces.

The mesenchymal cells lining the bony trabeculae were flattened and appeared inactive. Based on H&E and TRAP stains, osteoclasts were rarely found on the trabecular bone surfaces in the OPG expressors. In contrast, osteoclasts and/or chondroclasts were seen in the region of the growth plate resorbing cartilage, but their numbers may be reduced compared to controls. Also, osteoclasts were present on the cortical surface of the metaphysis where modelling activity is usually robust. The predominant difference between the expressors and controls was the profound decrease in trabecular osteoclasts, both in the vertebrae and femurs. The extent of bone accumulation was directly correlated with

the level of OPG transgene mRNA detected by northern blotting of total liver RNA.

The spleens from the OPG expressors had an increased amount of red pulp with the expansion due to increased hematopoiesis. All hematopoietic lineages are represented. F480 positive cells were present in both control and OPG expressors in the red pulp. Two of the expressors (2 and 17)had foci of extramedullary hematopoiesis within the liver and this is likely due to the osteopetrotic marrow.

There were no observable abnormalities in the thymus, lymph nodes, gastrointestinal tract, pancreato-hepatobiliary tract, respiratory tract, reproductive system, genito-urinary system, skin, nervous system, heart and aorta, breast, skeletal muscle and fat.

EXAMPLE 5

Isolation of mouse and human OPG cDNA

A cDNA clone corresponding to the 5' end of the mouse OPG mRNA was isolated from a mouse kidney cDNA library (Clontech) by PCR amplification. The oligonucleotides were derived from the rat OPG cDNA sequence and are shown below:

5'-ATCAAAGGCAGGGCATACTTCCTG-3' (SEQ ID NO:13)
5'-GTTGCACTCCTGTTTCACGGTCTG-3' (SEQ ID NO:14)

5'-CAAGACACCTTGAAGGGCCTGATG-3' (SEQ ID NO:15)
5'-TAACTTTTACAGAAGAGCATCAGC-3' (SEQ ID NO:16)

5'-AGCGCGGCCGCATGAACAAGTGGCTGTGCTGCG-3' (SEQ ID NO:17)
5'-AGCTCTAGAGAAACAGCCCAGTGACCATTCC-3' (SEQ ID NO:18)

The partial and full-length cDNA products obtained in this process were sequenced. The full-length product was digested with Not I and Xba I, then directionally cloned into the plasmid vector pRcCMV (Invitrogen). The resulting plasmid was named pRcCMV-Mu-OPG. The nucleotide sequence of the cloned product was compared to the rat OPG cDNA sequence. Over the 1300 bp region spanning the OPG LORF, the rat and mouse DNA sequences are approximately 88% identical. The mouse cDNA sequence contained a 401 aa LORF, which was compared to the rat OPG protein sequence and found to be ~94% identical without gaps. This indicates that the mouse cDNA sequence isolated encodes the murine OPG protein, and that the sequence and structure has been highly conserved throughout evolution. The mouse OPG protein sequence contains an identical putative signal peptide at its N-terminus, and all 4 potential sites of N-linked glycosylation are conserved.

A partial human OPG cDNA was cloned from a human kidney cDNA library using the following rat-specific oligonucleotides:

5'-GTG AAG CTG TGC AAG AAC CTG ATG-3' (SEQ ID NO:19)

5'-ATC AAA GGC AGG GCA TAC TTC CTG-3' (SEQ ID NO:20)

This PCR product was sequenced and used to design primers for amplifying the 3' end of the human cDNA using a human OPG genomic clone in lambda as template:

5'-TCCGTAAGAAACAGCCCAGTGACC-3' (SEQ ID NO:29)

5'-CAGATCCTGAAGCTGCTCAGTTTG-3' (SEQ ID NO:21)

The amplified PCR product was sequenced, and together with the 5' end sequence, was used to design 5' and 3' human-specific primers useful for amplifying the entire human OPG cDNA coding sequences:

5'-AGCGCGGCCGCGGGGACCACAATGAACAAGTTG-3' (SEQ ID NO:22)
5'-AGCTCTAGAATTGTGAGGAAACAGCTCAATGGC-3' (SEQ ID NO:23)

The full-length human PCR product was sequenced, then directionally cloned into the plasmid vector pRcCMV (Invitrogen) using Not I and Xba I. The resulting plasmid was named pRcCMV-human OPG. The nucleotide sequence of the cloned product was compared to the rat and mouse OPG cDNA sequences. Over the 1300 bp region spanning the OPG LORF, the rat and mouse DNA sequences are approximately 78-88% identical to the human OPG cDNA. The human OPG cDNA sequence also contained a 401 aa LORF, and it was compared to the rat and mouse protein sequences. The predicted human OPG protein is approximatlely 85% identical, and ~90% identical to the rat and mouse proteins, respectively. Sequence alignment of rat, mouse and human proteins show that they have been highly conserved during evolution. The human protein is predicted to have a N-terminal signal peptide, and 5 potential sites of N-linked glycosylation, 4 of which are conserved between the rat and mouse OPG proteins.

The DNA and predicted amino acid sequence of mouse OPG is shown in Figure 9A and 9B (SEQ ID NO:122). The DNA and predicted amino acid sequence of human OPG is shown in Figure 9C an 9D (SEQ ID NO:124). A

comparison of the rat, mouse and human OPG amino acid sequences is shown in Figure 9E and 9F.

Isolation of additional human OPG cDNA clones revealed the presence of a G to C base change at position 103 of the DNA sequence shown in Figure 9C. This nucleotide change results in substitution of an asparagine for a lysine at position 3 of the amino acid sequence shown in Figure 9C. The remainder of the sequence in clones having this change was identical to that in Figure 9C and 9D.

EXAMPLE 6

OPG three-dimensional structure modelling

The amino-terminal portion of OPG has homology to the extracellular portion of all known members of the TNFR superfamily (Figure 1C). The most notable motif in this region of TNFR-related genes is an ~40 amino acid, cysteine-rich repeat sequence which folds into distinct structures (Banner et al. Cell 73, 431-445 (1993)). This motif is usually displayed in four (range 3-6) tandem repeats (see Figure 1C), and is known to be involved in ligand binding (Beutler and van Huffel Science 264, 667-663 (1994)). Each repeat usually contains six interspaced cysteine residues, which are involved in forming three intradomain disulfide bonds, termed SS1, SS2, and SS3 (Banner et al., ibid). In some receptors, such as TNFR2, CD30 and CD40, some of the repeat domains contain only two intrachain disulfide bonds (SS1 and SS3).

The human OPG protein sequence was aligned to a TNFR1 extracellular domain profile using methods described by Lu thy, et al., ibid, and the results were

graphically displayed using the PrettyPlot program from the Wisconsin Package, version 8.1 (Genetics Computer Group, Madison, WI) (Figure 10). The alignment indicates a clear conservation of cysteine residues involved in formation of domains 1-4. This alignment was then used to construct a three-dimensional (3-D) model of the human OPG N-terminal domain using the known 3-D structure of the extracellular domain of p55 TNFR1 (Banner et al., ibid) as the template. To do this the atomic coordinates of the peptide backbone and side chains of identical residues were copied from the crystal structure coordinates of TNFR1. Following this, the remaining coordinates for the insertions and different side chains were generated using the LOOK program (Molecular Applications Group, Palo Alto, CA). The 3-D model was then refined by minimizing its conformational energy using LOOK.

By analogy with other TNFR family members, it is assumed that OPG binds to a ligand. For the purpose of modelling the interaction of OPG with its ligand, the crystal structure of TNF-β was used to simulate a 3-D representation of an "OPG ligand". This data was graphically displayed (see Figure 11) using Molscript (Kraulis, J. Appl. Cryst. 24, 946-950, 1991). A model for the OPG/ligand complex with 3 TNFβ and 3 OPG molecules was constructed where the relative positions of OPG are identical to TNFR1 in the crystal structure. This model was then used to find the residues of OPG that could interact with its ligand using the following approach: The solvent accessible area of all residues in the complex and one single OPG model were calculated. The residues that have different accessibility in the complex than in the monomer are likely to interact with the ligand.

The human and mouse OPG amino acid sequences were realigned using this information to highlight sequences comprising each of the cysteine rich domains 1-4 (Figure 12A and 12B). Each domain has individual structural characteristics which can be predicted:

Domain 1

Contains 4 cysteines involved in SS2 (C41 to C54) and SS3 (C44 to C62) disulfide bonds. Although no SS1 bond is evident based on disulfide bridges, the conserved tyrosine at position 28 is homologous to Y20 in TNFR1, which is known to be involved in interacting with H66 to aid in domain formation. OPG has a homologous histidine at position 75, suggesting OPG Y28 and H75 stack together in the native protein, as do the homologous residues in TNFR1. Therefore, both of these residues may indeed be important for biological activity, and N-terminal OPG truncations up to and beyond Y28 may have altered activity. In addition, residues E34 and K43 are predicted to interact with a bound ligand based on our 3-dimensional model.

Domain 2

Contains six cysteines and is predicted to contain SS1 (C65 to C80), SS2 (C83 to C98) and SS3 (C87 to C105) disulfide bonds. This region of OPG also contains an region stretching from P66-Q91 which aligns to the portion of TNFR1 domain 2 which forms close contacts with TNFβ (see above), and may interact with an OPG ligand. In particular residues P66, H68, Y69, Y70, T71, D72, S73, H75, T76, S77, D78, E79, L81, Y82, P85, V86, K88, E89, L90, and Q91 are predicted to interact with a bound ligand based on our structural data.

Domain 3

Contains 4 cysteines involved in SS1 (C107 to C 118) and SS3 (C124 to C142) disulfide bonds, but not an SS2 bond. Based on our structural data, residues E115, L118 and K119 are predicted in to interact with an OPG ligand.

Domain 4

Contains 4 cysteines involved in SS1 (C145 to C160) and SS3 (C166 to C185) disulfide bonds, but not an SS2 bond, similar to domain 3. Our structural data predict that E153 and S155 interact with an OPG ligand.

Thus, the predicted structural model for OPG identifies a number of highly conserved residues which are likely to be important for its biological activity.

EXAMPLE 7

Production of recombinant secreted OPG protein in mammalian cells

To determine if OPG is actually a secreted protein, mouse OPG cDNA was fused to the human IgG1 Fc domain as a tag (Capon et al. Nature 337, 525-531 (1989)), and expressed in human 293 fibroblasts. Fc fusions were carried out using the vector pFc-A3. pFc-A3 contains the region encoding the Fc portion of human immunoglobulin IgG-γ1 heavy chain (Ellison et al. ibid) from the first amino acid of the hinge domain (Glu-99) to the carboxyl terminus and is flanked by a 5'-NotI fusion site and 3'-SalI and XbaI sites. The plasmid was constructed by PCR amplification of the human spleen cDNA library (Clontech). PCR r actions were in a final

volume of 100 µl and employed 2 units of Vent DNA polymerase (New England Biolabs) in 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 µM $(NH_4)2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100 with 400 µM each dNTP and 1 ng of the cDNA library to be amplified together with 1 µM of each primer. Reactions were initiated by denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 73°C for 2 min. The 5' primer

5' ATAGCGGCCGCTGAGCCCAAATCTTGTGACAAAACTCAC 3' (SEQ ID NO:24)

incorporated a NotI site immediately 5' to the first residue (Glu-99) of the hinge domain of IgG-γ1. The 3' primer

5'-TCTAGAGTCGACTTATCATTTACCCGGAGACAGGGAGAGGCTCTT-3' (SEQ ID NO:25)

incorporated SalI and XbaI sites. The 717-bp PCR product was digested with NotI and SalI, isolated by electrophoresis through 1% agarose (FMC Corp.),purified by the Geneclean procedure (BIO 101, Inc.) and cloned into NotI, SalI-digested pBluescript II KS vector (Stratagene). The insert in the resulting plasmid, pFc-A3, was sequenced to confirm the fidelity of the PCR reaction.

The cloned mouse cDNA in plasmid pRcCMV-MuOPG was amplified using the following two sets of primer pairs:

Pair 1

5'-CCTCTGAGCTCAAGCTTCCGAGGACCACAATGAACAAG-3' (SEQ ID NO:26)

5'-CCTCTGCGGCCGCTAAGCAGCTTATTTTCACGGATTGAACCTG-3' (SEQ ID NO:27)

Pair 2

5'-CCTCTGAGCTCAAGCTTCCGAGGACCACAATGAACAAG-3' (SEQ ID NO:28)

5'-CCTCTGCGGCCGCTGTTGCATTTCCTTTCTG-3' (SEQ ID NO:30)

The first pair amplifies the entire OPG LORF, and creates a NotI restriction site which is compatible with the in-frame Not I site in Fc fusion vector pFcA3. pFcA3 was prepared by engineering a NotI restriction site 5' to aspartic acid reside 216 of the human IgG1 Fc cDNA. This construct introduces a linker which encodes two irrelevant amino acids which span the junction between the OPG protein and the IgG Fc region. This product, when linked to the Fc portion, would encode all 401 OPG residues directly followed by all 227 amino acid residues of the human IgG1 Fc region (F1.Fc). The second primer pair amplifies the DNA sequences encoding the first 180 amino acid residues of OPG, which encompasses its putative ligand binding domain. As above, the 3' primer creates an artificial Not I restriction site which fuses the C-terminal truncated OPG LORF at position threonine 180 directly to the IgG1 Fc domain (CT.fc).

The amino acid sequence junction linking OPG residue 401 and aseptic acid residue 221 of the human Fc region can be modified as follows: The DNA encoding residues 216-220 of the human Fc region can be deleted as described below, or the cysteine residue corresponding to C220 of the human Fc region can be mutated to either serine or alanine. OPF-Fc fusion protein encoded by these modifed vectors can be transfected into human 293 cells, or CHO cells, and recombinant OPG-Fc fusion protein purified as described below.

Both products were directionally cloned into the plasmid vector pCEP4 (Invitrogen). pCEP4 contains the Epstein-Barr virus origin of replication, and is capable of episomal replication in 293-EBNA-1 cells. The parent pCEP4, and pCEP4-Fl.Fc and pCEP4-CT.Fc vectors were lipofected into 293-EBNA-1 cells using the manufacturer's recommended methods. The transfected cells were then selected in 100 μg/ml hygromycin to select for vector expression, and the resulting drug-resistant mass cultures were grown to confluence. The cells were then cultured in serum-free media for 72 hr, and the conditioned media removed and analysed by SDS-PAGE. A silver staining of the polyacrylamide gel detects the major conditioned media proteins produced by the drug resistant 293 cultures. In the pCEP4-Fl.Fc and the pCEP4-CT.Fc conditioned media, unique bands of the predicted sizes were abundantly secreted (see Figures 13B and 13C). The full-length Fc fusion protein accumulated to a high concentration, indicating that it may be stable. Both Fc fusion proteins were detected by anti-human IgG1 Fc antibodies (Pierce) on western blots, indicating that they are recombinant OPG products.

The full length OPG-Fc fusion protein was purified by Protein-A column chromatography (Pierce) using the manufacturers recommended procedures. The protein was then subjected to N-terminal sequence analysis by automated Edman degradation as essentially described by Matsudaira et al. (J. Biol. Chem. 262, 10-35 (1987)). The following amino acid sequence was read after 19 cycles:

NH2-E T L P P K Y L H Y D P E T G H Q L L-CO2H
(SEQ ID NO:31)

This sequence was identical to the predicted mouse OPG amino acid sequence beginning at amino acid residue 22, suggesting that the natural mammalian leader cleavage site is between amino acid residues Q21-E22, not between Y31-D32 as originally predicted. The expression experiments performed in 293-EBNA cells with pCEP4-F1.Fc and pCEP4-CT.Fc demonstrate that OPG is a secreted protein, and may act systemically to bind its ligand.

Procedures similar to those used to construct and express the muOPG[22-180]-Fc and muOPG[22-401]-Fc fusions were employed for additional mouse and human OPG-Fc fusion proteins.

Murine OPG cDNA encoding amino acids 1-185 fused to the Fc region of human IgG1 [muOPG Ct(185).Fc] was constructed as follows. Murine OPG cDNA from plasmid pRcCMV Mu Osteoprotegerin (described in Example 5) was amplified using the following primer pair in a polymerase chain reaction as described above:

1333-82:

5'-TCC CTT GCC CTG ACC ACT CTT-3' (SEQ ID NO:32)

1333-80:

5'-CCT CTG CGG CCG CAC ACA CGT TGT CAT GTG TTG C-3' (SEQ ID NO:33)

This primer pair amplifies the murine OPG cDNA region encoding amino acid residues 63-185 (corresponding to bp 278-645) of the OPG reading frame as shown in Figure 9A. The 3' primer contains a Not I restriction site which is compatible with the in-frame Not I site of the Fc fusion vector pFcA3. The product also spans a unique EcoRI restriction site located at bp 436. The amplified PCR product was purified, cleaved

with NotI and EcoRI, and the resulting EcoRI-NotI restriction fragment was purified. The vector pCEP4 having the murine 1-401 OPG-Fc fusion insert was cleaved with EcoRI and NotI, purified, and ligated to the PCR product generated above. The resulting pCEP4-based expression vector encodes OPG residues 1-185 directly followed by all 227 amino acid residues of the human IgG1 Fc region. The murine OPG 1-185.Fc fusion vector was transfected into 293 cells, drug selected, and conditioned media was produced as described above. The resulting secreted murine OPG 1-185.Fc fusion product was purified by Protein-A column chromatography (Pierce) using the manufacturers recommended procedures.

Murine OPG DNA encoding amino acid residues 1-194 fused to the Fc region of human IgG1 (muOPG Ct(194).Fc) was constructed as follows. Mouse OPG cDNA from plasmid pRcCMV Mu-Osteoprotegerin was amplified using the following primer pairs:

1333-82:

5'-TCC CTT GCC CTG ACC ACT CTT-3' (SEQ ID NO:34)

1333-81:

5'-CCT CTG CGG CCG CCT TTT GCG TGG CTT CTC TGT T-3' (SEQ ID NO:35)

This primer pair amplifies the murine OPG cDNA region encoding amino acid residues 70-194 (corresponding to bp 298-672) of the OPG reading frame. The 3' primer contains a Not I restriction site which is compatible with the in-frame Not I site of the Fc fusion vector pFcA3. The product also spans a unique EcoRI restriction site located at bp 436. The amplified PCR product was cloned into the murine OPG[1-401] Fc fusion

vector as described above. The resulting pCEP4-based expression vector encodes OPG residues 1-194 directly followed by all 227 amino acid residues of the human IgG1 Fc region. The murine OPG 1-194.Fc fusion vector was transfected into 293 cells, drug selected, and conditioned media was produced. The resulting secreted fusion product was purified by Protein-A column chromatography (Pierce) using the manufacturers recommended procedures.

Human OPG DNA encoding amino acids 1-401 fused to the Fc region of human IgG1 was constructed as follows. Human OPG DNA in plasmid pRcCMV-hu osteoprotegerin (described in Example 5) was amplified using the following oligonucleotide primers:

1254-90:
5'CCT CTG AGC TCA AGC TTG GTT TCC GGG GAC CAC AAT G-3'
(SEQ ID NO:36)

1254-95:
5'-CCT CTG CGG CCG CTA AGC AGC TTA TTT TTA CTG AAT GG-3'
(SEQ ID NO:37)

The resulting PCR product encodes the full-length human OPG protein and creates a Not I restriction site which is compatible with the in-frame Not I site Fc fusion vector FcA3. The PCR product was directionally cloned into the plasmid vector pCEP4 as described above. The resulting expression vector encodes human OPG residues 1-401 directly followed by 227 amino acid residues of the human IgG1 Fc region. Conditioned media from transfected and drug selected cells was produced and the huOPG Fl.Fc fusion product was purified by

Protein-A column chromatography (Pierce) using the manufacturers recommended procedures.

Human OPG DNA encoding amino acid residues 1-201 fused to the Fc region of human IgG1 [huOPG Ct(201).Fc] was constructed as follows. The cloned human OPG cDNA from plasmid pRrCMV-hu osteoprotegerin was amplified by PCR using the following oligonucleotide primer pair:

1254-90:
5'-CCT CTG AGC TCA AGC TTG GTT TCC GGG GAC CAC AAT G-3' (SEQ ID NO:38)
1254-92:
5'-CCT CTG CGG CCG CCA GGG TAA CAT CTA TTC CAC-3' (SEQ ID NO:39)

This primer pair amplifies the human OPG cDNA region encoding amino acid residues 1-201 of the OPG reading frame, and creates a Not I restriction site at the 3' end which is compatable with the in-frame Not I site Fc fusion vector FcA3. This product, when linked to the Fc portion, encodes OPG residues 1-201 directly followed by all 221 amino acid residues of the human IgG1 Fc region. The PCR product was directionally cloned into the plasmid vector pCEP4 as described above. Conditioned media from transfected and drug selected cells was produced, and the hu OPG Ct(201).Fc fusion products purified by Protein-A column chromatography (Pierce) using the manufacturer's recommended procedures.

The following procedures were used to construct and express unfused mouse and human OPG.

A plasmid for mammalian expression of full-length murine OPG (residues 1-401) was generated by PCR amplification of the murine OPG cDNA insert from pRcCMV Mu-Osteoprotegerin and subcloned into the expression vector pDSRα (DeClerck et. atl. J. Biol. Chem. 266, 3893 (1991)). The following oligonucleotide primers were used:

1295-26:

5'-CCG AAG CTT CCA CCA TGA ACA AGT GGC TGT GCT GC-3' (SEQ ID NO:40)

1295-27:

5'-CCT CTG TCG ACT ATT ATA AGC AGC TTA TTT TCA CGG ATT G-3' (SEQ ID NO:41)

The murine OPG full length reading frame was amplified by PCR as described above. The PCR product was purified and digested with restriction endonucleases Hind III and Xba I (Boehringer Mannheim, Indianapolis, IN) under the manufacturers recommended conditions, then ligated to Hind III and Xba I digested pDSRα. Recombinant clones were detected by restriction endonuclease digestion, then sequenced to ensure no mutations were produced during the PCR amplification steps.

The resulting plasmid, pDSRα-muOPG was introduced into Chinese hamster ovary (CHO) cells by calcium mediated transfection (Wigler et al. Cell 11, 233 (1977)). Individual colonies were selected based upon expression of the dihydrofolate reductase (DHFR) gene in the plasmid vector and several clones were isolated. Expression of the murine OPG recombinant protein was monitored by western blot analysis of CHO

cell conditioned media. High expressing cells were selected, and OPG expression was further amplified by treatment with methotrexate as described (DeClerck et al., idid). Conditioned media from CHO cell lines was produced for further purification of recombinant secreted murine OPG protein.

A plasmid for mammalian expression of full-length human OPG (amino acids 1-401) was generated by subcloning the cDNA insert in pRcCMV-hu Osteoprotegerin directly into vector pDSRα (DeClerck et al., ibid). The pRcCMV-OPG plasmid was digested to completion with Not I, blunt ended with Klenow, then digested to completion with Xba I. Vector DNA was digested with Hind III, blunt ended with Klenow, then digested with Xba I, then ligated to the OPG insert. Recombinant plasmids were then sequenced to confirm proper orientation of the human OPG cDNA.

The resulting plasmid pDSRα-huOPG was introduced into Chinese hamster ovary (CHO) cells as described above. Individual colonies were selected based upon expression of the dihydrofolate reductase (DHFR) gene in the plasmid vector and several clones were isolated. Expression of the human OPG recombinant protein was monitored by western blot analysis of CHO cell conditioned media. High expressing clones were selected, and OPG expression was further amplified by treatment with methotrexate. Conditioned media from CHO cell lines expressing human OPG was produced for protein purification.

Expression vectors for murine OPG encoding residues 1-185 were constructed as follows. Murine OPG

cDNA from pRcCMV-Mu OPG was amplified using the following oligonucleotide primers:

1333-82:

5'-TCC CTT GCC CTG ACC ACT CTT-3' (SEQ ID NO:42)

1356-12:

5'-CCT CTG TCG ACT TAA CAC ACG TTG TCA TGT GTT GC-3' (SEQ ID NO:43)

This primer pair amplifies the murine OPG cDNA region encoding amino acids 63-185 of the OPG reading frame (bp 278-645) and contains an artificial stop codon directly after the cysteine codon (C185), which is followed by an artificial Sal I restriction endonuclease site. The predicted product contains an internal Eco RI restriction site useful for subcloning into a pre-existing vector. After PCR amplification, the resulting purified product was cleaved with Eco RI and Sal I restriction endonucleases, and the large fragment was gel purified. The purified product was then subcloned into the large restriction fragment of an Eco RI and Sal I digest of pBluescript-muOPG Fl.Fc described above. The resulting plasmid was digested with Hind III and Xho I and the small fragment was gel purified. This fragment, which contains a open reading frame encoding residues 1-185 was then subcloned into a Hind III and Xho I digest of the expression vector pCEP4. The resulting vector, pmuOPG [1-185], encodes a truncated OPG polypeptide which terminates at a cysteine residue located at position 185. Conditioned media from transfected and drug selected cells was produced as described above.

1333-82:

5'-TCC CTT GCC CTG ACC ACT CTT-3' (SEQ ID NO:44)

1356-13:

5'-CCT CTG TCG ACT TAC TTT TGC GTG GCT TCT CTG TT-3' (SEQ ID NO:45)

This primer pair amplifies the murine OPG cDNA region encoding amino acids 70-194 of the OPG reading frame (bp 298-672) and contains an artificial stop codon directly after the lysine codon (K194), which is followed by an artificial Sal I restriction endonuclease site. The predicted product contains an internal Eco RI restriction site useful for subcloning into a pre-existing vector. After PCR amplification, the resulting purified product was cleaved with Eco RI and Sal I restriction endonucleases, and the large fragment was gel purified. The purified product was then subcloned into the large restriction fragment of an Eco RI and Sal I digest of pBluescript-muOPG Fl.Fc described above. The resulting plasmid was digested with Hind III and Xho I and the small fragment was gel purified. This fragment, which contains a open reading frame encoding residues 1-185 was then subcloned into a Hind III and Xho I digest of the expression vector pCEP4. The resulting vector, pmuOPG [1-185], encodes a truncated OPG polypeptide which terminates at a lysine at position 194. Conditioned media from transfected and drug selected cells was produced as described above.

Several mutations were generated at the 5' end of the huOPG [22-401]-Fc gene that introduce either amino acid substitutions, or deletions, of OPG between residues 22 through 32. All mutations were generated

with the "QuickChange™ Site-Directed Mutagenesis Kit" (Stratagene, San Diego, CA) using the manfacturer's recommended conditions. Briefly, reaction mix containing huOPG [22-401]-Fc plasmid DNA template and mutagenic primers were treated with Pfu polymerase in the presence of deoxynucleotides, then amplified in a thermocycler as described above. An aliqout of the reaction is then transfected into competent E. coli XL1-Blue by heatshock, then plated. Plasmid DNA from transformants was then sequenced to verify mutations.

The following primer pairs were used to delete residues 22-26 of the human OPG gene, resulting in the production of a huOPG [27-401]-Fc fusion protein:

1436-11:

5'-TGG ACC ACC CAG AAG TAC CTT CAT TAT GAC-3'
(SEQ ID NO:140)

1436-12:

5'-GTC ATA ATG AAG GTA CTT CTG GGT GGT CCA-3'
(SEQ ID NO:141)

The following primer pairs were used to delete residues 22-28 of the human OPG gene, resulting in the production of a huOPG [29-401]-Fc fusion protein:

1436-17:

5'-GGA CCA CCC AGC TTC ATT ATG ACG AAG AAA C-3'
(SEQ ID NO:142)

1436-18:

5'-GTT TCT TCG TCA TAA TGA AGC TGG GTG GTC C-3'
(SEQ ID NO:143)

The following primer pairs were used to delete residues 22-31 of the human OPG gene, resulting in the production of a huOPG [32-401]-Fc fusion protein:

1436-27:

5'-GTG GAC CAC CCA GGA CGA AGA AAC CTC TC-3'
(SEQ ID NO:144)

1436-28:

5'-GAG AGG TTT CTT CGT CCT GGG TGG TCC AC-3'
(SEQ ID NO:145)

The following primer pairs were used to change the codon for tyrosine residue 28 to phenylalanine of the human OPG gene, resulting in the production of a huOPG [22-401]-Fc Y28F fusion protein:

1436-29:

5'-CGT TTC CTC CAA AGT TCC TTC ATT ATG AC-3'
(SEQ ID NO:146)

1436-30:

5'-GTC ATA ATG AAG GAA CTT TGG AGG AAA CG-3'
(SEQ ID NO:147)

The following primer pairs were used to change the codon for proline residue 26 to alanine of the human OPG gene, resulting in the production of a huOPG [22-401]-Fc P26A fusion protein:

1429-83:

5'-GGA AAC GTT TCC TGC AAA GTA CCT TCA TTA TG-3
(SEQ ID NO:148)

1429-84:

5'-CAT AAT GAA GGT ACT TTG CAG GAA ACG TTT CC-3'

(SEQ ID NO:149)

Each resulting muOPG [22-401]-Fc plasmid containing the appropriate mutation was then transfected into human 293 cells, the mutant OPG-Fc fusion protein purified from conditioned media as described above. The biological activity of each protein was assessed the in vitro osteoclast forming assay described in Example 11.

EXAMPLE 8

Expression of OPG in E. coli

A. Bacterial Expression Vectors

pAMG21

The expression plasmid pAMG21 can be derived from the Amgen expression vector pCFM1656 (ATCC #69576) which in turn be derived from the Amgen expression vector system described in US Patent No. 4,710,473. The pCFM1656 plasmid can be derived from the described pCFM836 plasmid (Patent No. 4,710,473) by: (a) destroying the two endogenous NdeI restriction sites by end filling with T4 polymerase enzyme followed by blunt end ligation; (b) replacing the DNA sequence between the unique AatII and ClaI restriction sites containing the synthetic P_L promoter with a similar fragment obtained from pCFM636 (patent No. 4,710,473) containing the PL promoter

```
 AatII
5'     CTAATTCCGCTCTCACCTACCAAACAATGCCCCCCTGCAAAAAATAAATTCATAT-
3'TGCAGATTAAGGCGAGAGTGGATGGTTTGTTACGGGGGGACGTTTTTTATTTAAGTATA-

-AAAAAACATACAGATAACCATCTGCGGTGATAAATTATCTCTGGCGGTGTTGACATAAA-
-TTTTTTGTATGTCTATTGGTAGACGCCACTATTTAATAGAGACCGCCACAACTGTATTT-

-TACCACTGGCGGTGATACTGAGCACAT  3' (SEQ ID NO:53)
-ATGGTGACCGCCACTATGACTCGTGTAGC5' (SEQ ID NO:54)
                          ClaI
```

and then (c) substituting the small DNA sequence between the unique *ClaI* and *KpnI* restriction sites with the following oligonucleotide:

```
5'     CGATTTGATTCTAGAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGGTAC3'
(SEQ ID NO:48)
3'       TAAACTAAGATCTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGC     5'
(SEQ ID NO:49)
       ClaI                                                KpnI
```

The expression plasmid pAMG21 can then be derived from pCFM1656 by making a series of site directed base changes by PCR overlapping oligo mutagenesis and DNA sequence substitutions. Starting with the BglII site (plasmid bp # 180) immediately 5' to the plasmid replication promoter PcopB and proceeding toward the plasmid replication genes, the base pair changes are as follows:

pAMG21 bp #	bp in pCFM1656	bp changed to in pAMG21
# 204	T/A	C/G
# 428	A/T	G/C
# 509	G/C	A/T
# 617	- -	insert two G/C bp
# 679	G/C	T/A
# 980	T/A	C/G
# 994	G/C	A/T
# 1004	A/T	C/G

# 1007	C/G	T/A
# 1028	A/T	T/A
# 1047	C/G	T/A
# 1178	G/C	T/A
# 1466	G/C	T/A
# 2028	G/C	bp deletion
# 2187	C/G	T/A
# 2480	A/T	T/A
# 2499-2502	AGTG TCAC	GTCA CAGT
# 2642	TCCGAGC AGGCTCG	7 bp deletion
# 3435	G/C	A/T
# 3446	G/C	A/T
# 3643	A/T	T/A

The DNA sequence between the unique AatII (position #4364 in pCFM1656) and SacII (position #4585 in pCFM1656) restriction sites is substituted with the following DNA sequence:

```
[AatII sticky end]           5'     GCGTAACGTATGCATGGTCTCC-
(position #4358 in pAMG21)   3' TGCACGCATTGCATACGTACCAGAGG-

-CCATGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT-
-GGTACGCTCTCATCCCTTGACGGTCCGTAGTTTATTTTGCTTTCCGAGTCAGCTTTCTGA-

-GGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGC-
-CCCGGAAAGCAAAATAGACAACAAACAGCCACTTGCGAGAGGACTCATCCTGTTTAGGCG-

-CGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGC-
-GCCCTCGCCTAAACTTGCAACGCTTCGTTGCCGGGCCTCCCACCGCCCGTCCTGCGGGCG-

-CATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGT-
-GTATTTGACGGTCCGTAGTTTAATTCGTCTTCCGGTAGGACTGCCTACCGGAAAAACGCA-

                                                    AatII
-TTCTACAAACTCTTTTGTTTATTTTTCTAAATACATTCAAATATGGACGTCGTACTTAAC-
-AAGATGTTTGAGAAAACAAATAAAAAGATTTATGTAAGTTTATACCTGCAGCATGAATTG-

-TTTTAAAGTATGGGCAATCAATTGCTCCTGTTAAAATTGCTTTAGAAATACTTTGGCAGC-
-AAAATTTCATACCCGTTAGTTAACGAGGACAATTTTAACGAAATCTTTATGAAACCGTCG-

-GGTTTGTTGTATTGAGTTTCATTTGCGCATTGGTTAAATGGAAAGTGACCGTGCGCTTAC-
-CCAAACAACATAACTCAAAGTAAACGCGTAACCAATTTACCTTTCACTGGCACGCGAATG-
```

-TACAGCCTAATATTTTTGAAATATCCCAAGAGCTTTTTCCTTCGCATGCCCACGCTAAAC-
-ATGTCGGATTATAAAAACTTTATAGGGTTCTCGAAAAAGGAAGCGTACGGGTGCGATTTG-

-ATTCTTTTTCTCTTTTGGTTAAATCGTTGTTTGATTTATTATTTGCTATATTTATTTTTC-
-TAAGAAAAAGAGAAAACCAATTTAGCAACAAACTAAATAATAAACGATATAAATAAAAAG-

-GATAATTATCAACTAGAGAAGGAACAATTAATGGTATGTTCATACACGCATGTAAAAATA-
-CTATTAATAGTTGATCTCTTCCTTGTTAATTACCATACAAGTATGTGCGTACATTTTTAT-

-AACTATCTATATAGTTGTCTTTCTCTGAATGTGCAAAACTAAGCATTCCGAAGCCATTAT-
-TTGATAGATATATCAACAGAAAGAGACTTACACGTTTTGATTCGTAAGGCTTCGGTAATA-

-TAGCAGTATGAATAGGGAAACTAAACCCAGTGATAAGACCTGATGATTTCGCTTCTTTAA-
-ATCGTCATACTTATCCCTTTGATTTGGGTCACTATTCTGGACTACTAAAGCGAAGAAATT-

-TTACATTTGGAGATTTTTTATTTACAGCATTGTTTTCAAATATATTCCAATTAATCGGTG-
-AATGTAAACCTCTAAAAAATAAATGTCGTAACAAAAGTTTATATAAGGTTAATTAGCCAC-

-AATGATTGGAGTTAGAATAATCTACTATAGGATCATATTTTATTAAATTAGCGTCATCAT-
-TTACTAACCTCAATCTTATTAGATGATATCCTAGTATAAAATAATTTAATCGCAGTAGTA-

-AATATTGCCTCCATTTTTTAGGGTAATTATCCAGAATTGAAATATCAGATTTAACCATAG-
-TTATAACGGAGGTAAAAAATCCCATTAATAGGTCTTAACTTTATAGTCTAAATTGGTATC-

-AATGAGGATAAATGATCGCGAGTAAATAATATTCACAATGTACCATTTTAGTCATATCAG-
-TTACTCCTATTTACTAGCGCTCATTTATTATAAGTGTTACATGGTAAAATCAGTATAGTC-

-ATAAGCATTGATTAATATCATTATTGCTTCTACAGGCTTTAATTTTATTAATTATTCTGT-
-TATTCGTAACTAATTATAGTAATAACGAAGATGTCCGAAATTAAAATAATTAATAAGACA-

-AAGTGTCGTCGGCATTTATGTCTTTCATACCCATCTCTTTATCCTTACCTATTGTTTGTC-
-TTCACAGCAGCCGTAAATACAGAAAGTATGGGTAGAGAAATAGGAATGGATAACAAACAG-

-GCAAGTTTTGCGTGTTATATATCATTAAAACGGTAATAGATTGACATTTGATTCTAATAA-
-CGTTCAAAACGCACAATATATAGTAATTTTGCCATTATCTAACTGTAAACTAAGATTATT-

-ATTGGATTTTTGTCACACTATTATATCGCTTGAAATACAATTGTTTAACATAAGTACCTG-
-TAACCTAAAAACAGTGTGATAATATAGCGAACTTTATGTTAACAAATTGTATTCATGGAC-

-TAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCGATTAATCGATTTGATT-
-ATCCTAGCATGTCCAAATGCGTTCTTTTACCAAACAATATCAGCTAATTAGCTAAACTAA-

-CTAGATTTGTTTTAACTAATTAAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGA-
-GATCTAAACAAAATTGATTAATTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGCT-

SacII
-GCTCACTAGTGTCGACCTGCAGGGTACCATGGAAGCTTACTCGAGGATCCGCGGAAAGAA-
-CGAGTGATCACAGCTGGACGTCCCATGGTACCTTCGAATGAGCTCCTAGGCGCCTTTCTT-

-GAAGAAGAAGAAGAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATA-
-CTTCTTCTTCTTCTTTCGGGCTTTCCTTCGACTCAACCGACGACGGTGGCGACTCGTTAT-

-ACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGG-
-TGATCGTATTGGGGAACCCCGGAGATTTGCCCAGAACTCCCCAAAAAACGACTTTCCTCC-

-AACCGCTCTTCACGCTCTTCACGC 3' [SacII sticky end] (SEQ ID NO:50)
-TTGGCGAGAAGTGCGAGAAGTG 5' (position #5904 in pAMG21) (SEQ ID NO:46)

During the ligation of the sticky ends of this substitution DNA sequence, the outside AatII and SacII sites are destroyed. There are unique AatII and SacII sites in the substituted DNA.

pAMG22-His

The expression plasmid pAMG22-His can be derived from the Amgen expression vector pAMG22 by substituting the small DNA sequence between the unique NdeI (#4795) and EcoRI (#4818) restriction sites of pAMG22 with the following oligonucleotide duplex:

```
  NdeI                               NheI           EcoRI
5' TATGAAACATCATCACCATCACCATCATGCTAGCGTTAACGCGTTGG    3'
(SEQ ID NO:51)
3'   ACTTTGTAGTAGTGGTAGTGGTAGTACGATCGCAATTGCGCAACCTTAA  5'
(SEQ ID NO:52)
     MetLysHisHisHisHisHisHisHisAlaSerValAsnAlaLeuGlu
(SEQ ID NO:168)
```

pAMG22

The expression plasmid pAMG22 can be derived from the Amgen expression vector pCFM1656 (ATCC #69576) which in turn be derived from the Amgen expression vector system described in US Patent No. 4,710,473 granted December 1, 1987. The pCFM1656 plasmid can be derived from the described pCFM836 plasmid (Patent No. 4,710,473) by: (a) destroying the two endogenous NdeI restriction sites by end filling with T4 polymerase enzyme followed by blunt end ligation; (b) replacing the DNA sequence between the unique AatII and ClaI restriction sites

containing the synthetic PL promoter with a similar fragment obtained from pCFM636 (patent No. 4,710,473) containing the PL promoter

AatII

5'   CTAATTCCGCTCTCACCTACCAAACAATGCCCCCCTGCAAAAAATAAATTCATAT-

3' TGCAGATTAAGGCGAGAGTGGATGGTTTGTTACGGGGGGACGTTTTTTATTTAAGTATA-

-AAAAAACATACAGATAACCATCTGCGGTGATAAATTATCTCTGGCGGTGTTGACATAAA-

-TTTTTTGTATGTCTATTGGTAGACGCCACTATTTAATAGAGACCGCCACAACTGTATTT-

-TACCACTGGCGGTGATACTGAGCACAT 3' (SEQ ID NO:53)

-ATGGTGACCGCCACTATGACTCGTGTAGC5' (SEQ ID NO:54)

ClaI

and then (c) substituting the small DNA sequence between the unique *ClaI* and *KpnI* restriction sites with the following oligonucleotide:

5' CGATTTGATTCTAGAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGGTAC 3' (SEQ ID NO:55)

3'   TAAACTAAGATCTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGC   5' (SEQ ID NO:56)

ClaI KpnI

The expression plasmid pAMG22 can then be derived from pCFM1656 by making a series of site directed base changes by PCR overlapping oligo mutagenesis and DNA sequence substitutions. Starting with the BglII site (plasmid bp # 180) immediately 5' to the plasmid replication promoter PcopB and proceeding toward the plasmid replication genes, the base pair changes are as follows:

pAMG22 bp #	bp in pCFM1656	bp changed to in pAMG22
# 204	T/A	C/G
# 428	A/T	G/C
# 509	G/C	A/T
# 617	- -	insert two G/C bp
# 679	G/C	T/A
# 980	T/A	C/G
# 994	G/C	A/T
# 1004	A/T	C/G
# 1007	C/G	T/A
# 1028	A/T	T/A
# 1047	C/G	T/A
# 1178	G/C	T/A
# 1466	G/C	T/A
# 2028	G/C	bp deletion
# 2187	C/G	T/A
# 2480	A/T	T/A
# 2499-2502	AGTG TCAC	GTCA CAGT
# 2642	TCCGAGC AGGCTCG	7 bp deletion
# 3435	G/C	A/T
# 3446	G/C	A/T
# 3643	A/T	T/A

The DNA sequence between the unique AatII (position #4364 in pCFM1656) and *SacII* (position #4585 in pCFM1656) restriction sites is substituted with the following DNA sequence:

[AatII sticky end] (position #4358 in pAMG22)

```
5'     GCGTAACGTATGCATGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAA-
3' TGCACGCATTGCATACGTACCAGAGGGGTACGCTCTCATCCCTTGACGGTCCGTAGTT-

-ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTG-
-TATTTTGCTTTCCGAGTCAGCTTTCTGACCCGGAAAGCAAAATAGACAACAAACAGCCAC-
```

```
-AACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGG-
-TTGCGAGAGGACTCATCCTGTTTAGGCGGCCCTCGCCTAAACTTGCAACGCTTCGTTGCC-

-CCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAG-
-GGGCCTCCCACCGCCCGTCCTGCGGGCGGTATTTGACGGTCCGTAGTTTAATTCGTCTTC-

-GCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTTTGTTTATTTTTCTAAAT-
-CGGTAGGACTGCCTACCGGAAAAACGCAAAGATGTTTGAGAAAACAAATAAAAAGATTTA-

              AatII
-ACATTCAAATATGGACGTCTCATAATTTTTAAAAAATTCATTTGACAAATGCTAAAATTC-
-TGTAAGTTTATACCTGCAGAGTATTAAAAATTTTTTAAGTAAACTGTTTACGATTTTAAG-

-TTGATTAATATTCTCAATTGTGAGCGCTCACAATTTATCGATTTGATTCTAGATTTGTTT-
-AACTAATTATAAGAGTTAACACTCGCGAGTGTTAAATAGCTAAACTAAGATCTAAACTCA-

-TAACTAATTAAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGAGCTCACTAGTGT-
-ATTGATTAATTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGCTCGAGTGATCACA-

                                                 SacII
-CGACCTGCAGGGTACCATGGAAGCTTACTCGAGGATCCGCGGAAAGAAGAAGAAGAAGAA-
-GCTGGACGTCCCATGGTACCTTCGAATGAGCTCCTAGGCGCCTTTCTTCTTCTTCTTCTT-

-GAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACC-
-CTTTCGGGCTTTCCTTCGACTCAACCGACGACGGTGGCGACTCGTTATTGATCGTATTGG-

-CCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACCGCTCTTCA-
-GGAACCCCGGAGATTTGCCCAGAACTCCCCAAAAAACGACTTTCCTCCTTGGCGAGAAGT-

-CGCTCTTCACGC 3' (SEQ ID NO:58)
-GCGAGAAGTG   5' (SEQ ID NO:57)

        [SacII sticky end] (position #5024 in pAMG22)
```

During the ligation of the sticky ends of this substitution DNA sequence, the outside AatII and SacII sites are destroyed. There are unique AatII and SacII sites in the substituted DNA.

B. Human OPG Met[32-401]

In the example, the expression vector used was pAMG21, a derivative of pCFM1656 (ATCC accession no. 69576) which contains appropriate restriction sites for insertion of genes downstream from the lux PR promoter. (See U.S. Patent No. 5,169,318 for description of the lux expression system). The host cell used was GM120

(ATCC accession no. 55764). This host has the lacIQ promoter and lacI gene integrated into a second site in the host chromosome of a prototrophic *E. coli* K12 host. Other commonly used *E. coli* expression vectors and host cells are also suitable for expression.

A DNA sequence coding for an N-terminal methionine and amino acids 32-401 of the human OPG polypeptide was placed under control of the luxPR promoter in the plasmid expression vector pAMG21 as follows. To accomplish this, PCR using oligonucleotides #1257-20 and #1257-19 as primers was performed using as a template plasmid pRcCMV-Hu OPG DNA containing the human OPG cDNA and thermocycling for 30 cycles with each cycle being: 94°C for 20 seconds, followed by 37°C for 30 seconds, followed by 72°C for 30 seconds. The resulting PCR sample was resolved on an agarose gel, the PCR product was excised, purified, and restricted with KpnI and BamHI restriction endonucleases and purified. Synthetic oligonucleotides #1257-21 and #1257-22 were phophorylated individually using T4 polynucleotide kinase and ATP, and were then mixed together, heated at 94°C and allowed to slow cool to room temperature to form an oligonucleotide linker duplex containing NdeI and KpnI sticky ends. The phosphorylated linker duplex formed between oligonucleotides #1257-21 and #1257-22 containing NdeI and KpnI cohesive ends (see Figure 14A) and the KpnI and BamHI digested and purified PCR product generated using oligo primers #1257-20 and #1257-19 (see above) was directionally inserted between two sites of the plasmid vector pAMG21, namely the NdeI site and BamHI site, using standard recombinant DNA methodology (see Figure 14A and sequences below). The synthetic linker utilized *E. coli* codons and provided for a N-terminal methionine.

Two clones were selected and plasmid DNA isolated, and the human OPG insert was subsequently DNA sequence confirmed. The resulting pAMG21 plasmid containing amino acids 32-401 of the human OPG polypeptide immediately preceded in frame by a methionine is referred to as pAMG21-huOPG met[32-401] or pAMG21-huOPG met[32-401].

Oligo#1257-19
5'-TACGCACTGGATCCTTATAAGCAGCTTATTTTTACTGATTGGAC-3'
(SEQ ID NO:59)

Oligo#1257-20
5'-GTCCTCCTGGTACCTACCTAAAACAAC-3' (SEQ ID NO:60)

Oligo#1257-21
5'-TATGGATGAAGAAACTTCTCATCAGCTGCTGTGTGATAAATGTCC
GCCGGGTAC -3' (SEQ ID NO:61)

Oligo#1257-22
5'-CCGGCGGACATTTATCACACAGCAGCTGATGAGAAGTTTCTTCATCCA-3'
(SEQ ID NO:47)

Cultures of pAMG21-huOPG met[32-401] in E. coli GM120 in 2XYT media containing 20 μg/ml kanamycin were incubated at 30°C prior to induction. Induction of huOPG met[32-401] gene product expression from the luxPR promoter was achieved following the addition of the synthetic autoinducer N-(3-oxohexanoyl)-DL-homoserine lactone to the culture media to a final concentration of 30 ng/ml and cultures were incubated at either 30°C or 37°C for a further 6 hours. After 6 hours, the bact rial cultures were examined by microscopy for the presence of inclusion bodies and were then pelletted by

centrifugation. Refractile inclusion bodies were observed in induced cultures indicating that some of the recombinant huOPG met[32-401] gene product was produced insolubly in E. coli. Some bacterial pellets were resuspended in 10mM Tris-HCl/pH8, 1mM EDTA and lysed directly by addition of 2X Laemlli sample buffer to 1X final, and β-mercaptoethanol to 5% final concentration, and analyzed by SDS-PAGE. A substantially more intense coomassie stained band of approximately 42kDa was observed on a SDS-PAGE gel containing total cell lysates of 30°C and 37°C induced cultures versus lane 2 which is a total cell lysate of a 30°C uninduced culture (Figure 14B). The expected gene product would be 370 amino acids in length and have an expected molecular weight of about 42.2 kDa. Following induction at 37°C for 6 hours, an additional culture was pelleted and either processed for isolation of inclusion bodies (see below) or processed by microfluidizing. The pellet processed for microfluidizing was resuspended in 25mM Tris-HCl/pH8, 0.5M NaCl buffer and passed 20 times through a Microfluidizer Model 1108 (Microfluidics Corp.) and collected. An aliquot was removed of the collected sample (microfluidized total lysate), and the remainder was pelleted at 20,000 x g for 20 minutes. The supernatant following centrifugation was removed (microfluidized soluble fraction) and the pellet resuspended in a 25mM Tris-HCl/pH8, 0.5M NaCl, 6M urea solution (microfluidized insoluble fraction). To an aliquot of either the total soluble, or insoluble fraction was added to an equal volume of 2X Laemalli sample buffer and β-mercaptoethanol to 5% final concentration. The samples were then analyzed by SDS-PAGE. A significant amount of recombinant huOPG met[32-401] gene product appeared to be found in the

insoluble fraction. To purify the recombinant protein inclusion bodies were purified as follows: Bacterial cells were separated from media by density gradient centrifugation in a Beckman J-6B centrifuge equipped with a JS-4.2 rotor at 4,900 x g for 15 minutes at 4°C. The bacterial pellet was resuspended in 5 ml of water and then diluted to a final volume of 10 ml with water. This suspension was transferred to a stainless steel cup cooled in ice and subjected to sonic disruption using a Branson Sonifier equipped with a standard tip (power setting=5, duty cycle=95%, 80 bursts). The sonicated cell suspension was centrifuged in a Beckman Optima TLX ultracentrifuge equipped with a TLA 100.3 rotor at 195,000 x g for 5 to 10 minutes at 23°C. The supernatant was discarded and the pellet rinsed with a stream of water from a squirt bottle. The pellets were collected by scraping with a micro spatula and transferred to a glass homogenizer (15 ml capacity). Five ml of Percoll solution (75% liquid Percoll, 0.15 M sodium chloride) was added to the homogenizer and the contents are homogenized until uniformly suspended. The volume was increased to 19.5 ml by the addition of Percoll solution, mixed, and distributed into 3 Beckman Quick-Seal tubes (13 x 32 mm). Tubes were sealed according to manufacturers instructions. The tubes were spun in a Beckman TLA 100.3 rotor at 23°C, 20,000 rpm (21,600 x g), 30 minutes. The tubes were examined for the appropriate banding pattern. To recover the refractile bodies, gradient fractions were recovered and pooled, then diluted with water. The inclusion bodies were pelleted by centrifugation, and the protein concentration estimated following SDS-PAGE.

An aliquot of inclusion bodies isolated as described below was dissolved into 1X Laemlli sample

buffer with 5% β-mercaptoethanol and resolved on a SDS-PAGE gel and the isolated inclusion bodies provide a highly purified recombinant huOPG[32-401] gene product. The major ~42 kDa band observed after resolving inclusion bodies on a SDS-polyacrylamide gel was excised from a separate gel and the N-terminal amino acid sequence determined essentially as described (Matsudaira et al. J. Biol. Chem. 262, 10-35 (1987)). The following sequence was determined after 19 cycles:

NH_2 -MDEETSHQLLCDKCPPGTY-COOH (SEQ ID NO:62)

This sequence was found to be identical to the first 19 amino acids encoded by the pAMG21 Hu-OPG met[32-401] expression vector, produced by a methionine residue provided by the bacterial expression vector.

C. Human OPG met[22-401]

A DNA sequence coding for an N-terminal methionine and amino acids 22 through 401 of human OPG was placed under control of the luxPR promoter in a prokaryotic plasmid expression vector pAMG21 as follows. Isolated plasmid DNA of pAMG21-huOPG met[32-401] (see Section B) was cleaved with KpnI and BamHI restriction endonucleases and the resulting fragments were resolved on an agarose gel. The B fragment (~1064 bp fragment) was isolated from the gel using standard methodology. Synthetic oligonucleotides (oligos) #1267-06 and #1267-07 were phosphorylated individually and allowed to form an oligo linker duplex, which contained NdeI and KpnI cohesive ends, using methods described in Section B. The synthetic linker duplex utilized *E. coli* codons and provided for an N-terminal methionine. The phosphorylated oligo linker containing NdeI and KpnI

cohesive ends and the isolated ~1064 bp fragment of pAMG21-huOP met[32-401] digested with KpnI and BamHI restriction endonucleases were directionally inserted between the NdeI and BamHI sites of pAMG21 using standard recombinant DNA methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the huOPG-met[22-401] gene.

Oligo #1267-06

5'-TAT GGA AAC TTT TCC TCC AAA ATA TCT TCA TTA TGA TGA AGA AAC TTC TCA TCA GCT GCT GTG TGA TAA ATG TCC GCC GGG TAC-3' (SEQ ID NO:63)

Oligo #1267-07

5'-CCG GCG GAC ATT TAT CAC ACA GCA GCT GAT GAG AAG TTT CTT CAT CAT AAT GAA GAT ATT TTG GAG GAA AAG TTT CCA-3' (SEQ ID NO:64)

Cultures of pAMG21-huOPG-met[22-401] in E. coli host 393 were placed in 2XYT media containing 20 µg/ml kanamycin and were incubated at 30°C prior to induction. Induction of recombinant gene product expression from the luxPR promoter of vector pAMG21 was achieved following the addition of the synthetic autoinducer N-(3-oxohexanoyl)-DL-homoserine lactone to the culture media to a final concentration of 30 ng/ml and incubation at either 30°C or 37°C for a further 6 hours. After 6 hours, bacterial cultures were pelleted by centrifugation (=30°C I+6 or 37°C I+6). Bacterial cultures were also either pelleted just prior to induction (=30°C PreI) or alternatively no autoinducer

was added to a separate culture which was allowed to incubate at 30°C for a further 6 hours to give an uninduced (UI) culture (=30°C UI). Bacterial pellets of either 30°C PreI, 30°C UI, 30°C I+6, or 37°C I+6 cultures were resuspended, lysed, and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) as described in Section B. Polyacrylamide gels were either stained with coomassie blue and/or Western transferred to nitrocellulose and immunoprobed with rabbit anti-mu OPG-Fc polyclonal antibody as described in Example 10. The level of gene product following induction compared to either an uninduced (30°C UI) or pre-induction (30°C PreI) sample.

D. Murine OPG met[22-401]

A DNA sequence coding for an N-terminal methionine and amino acids 22 through 401 of the murine (mu) OPG (OPG) polypeptide was placed under control of the luxPR promoter in a prokaryotic plasmid expression vector pAMG21 as follows. PCR was performed using oligonucleotides #1257-16 and #1257-15 as primers, plasmid pRcCMV-Mu OPG DNA as a template and thermocycling conditions as described in Section B. The PCR product was purified and cleaved with KpnI and BamHI restriction endonucleases as described in Section B. Synthetic oligos #1260-61 and #1260-82 were phosphorylated individually and allowed to form an oligo linker duplex with NdeI and KpnI cohesive ends using methods described in Section B. The synthetic linker duplex utilized *E. coli* codons and provided for an N-terminal methionine. The phosphorylated linker duplex formed between oligos #1260-61 and #1260-82 containing NdeI and KpnI cohesive ends and the KpnI and BamHI digested and purified PCR product generated using oligo

primers #1257-16 and #1257-15 were directionally inserted between the NdeI and BamHI sites of pAMG21 using standard methodology. The ligation mixture was transformed into *E. coli* host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the MuOPG met[22-401] gene.

Expression of recombinant muOPG met[22-401] polypeptide from cultures of 393 cells harboring plasmid pAMG21-MuOPG met[22-401] following induction was determined using methods described in Section C.

Oligo #1257-15
5'-TAC GCA CTG GAT CCT TAT AAG CAG CTT ATT TTC ACG GAT TGA AC-3' (SEQ ID NO:65)

Oligo #1257-16
5'-GTG CTC CTG GTA CCT ACC TAA AAC AGC ACT GCA CAG TG-3' (SEQ ID NO:66)

Oligo #1260-61
5'-TAT GGA AAC TCT GCC TCC AAA ATA CCT GCA TTA CGA TCC GGA AAC TGG TCA TCA GCT GCT GTG TGA TAA ATG TGC TCC GGG TAC-3' (SEQ ID NO:67)

Oligo #1260-82
5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CCG GAT CGT AAT GCA GGT ATT TTG GAG GCA GAG TTT CCA-3' (SEQ ID NO:68)

E. Murine OPG met[32-401]

A DNA sequence coding for an N-terminal methionine and amino acids 32 through 401 of murine OPG

was placed under control of the luxPR promoter in a prokaryotic plasmid expression vector pAMG21 as follows. To accomplish this, Synthetic oligos #1267-08 and #1267-09 were phosphorylated individually and allowed to form an oligo linker duplex using methods described in Section B. The synthetic linker duplex utilized E. coli codons and provided for an N-terminal methionine. The phosphorylated linker duplex formed between oligos #1267-08 and #1267-09 containing NdeI and KpnI cohesive ends, and the KpnI and BamHI digested and purified PCR product described earlier (see Section D), was directionally inserted between the NdeI and BamHI sites of pAMG21 using standard methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the muOPG-met[32-401] gene.

Expression of recombinant muOPG-met [32-401] polypeptide from cultures of 393 cells harboring the pAMG21 recombinant plasmid following induction was determined using methods described in Section C.

Oligo #1267-08

5'-TAT GGA CCC AGA AAC TGG TCA TCA GCT GCT GTG TGA TAA ATG TGC TCC GGG TAC-3' (SEQ ID NO:69)

Oligo #1267-09

5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CTG GGT CCA-3' (SEQ ID NO:70)

E. Murine OPG met-lys[22-401]

A DNA sequence coding for an N-terminal methionine followed by a lysine residue and amino acids 22 through 401 of murine OPG was placed under control of the lux PR promoter in prokaryotic expression vector pAMG21 as follows. Synthetic oligos #1282-95 and #1282-96 were phosphorylated individually and allowed to form an oligo linker duplex using methods described in Section B. The synthetic linker duplex utilized E. coli codons and provided for an N-terminal methionine. The phosphorylated linker duplex formed between oligos #1282-95 and #1282-96 containing NdeI and KpnI cohesive ends and the KpnI and BamHI digested and purified PCR product described in Section D was directionally inserted between the NdeI and BamHI sites in pAMG21 using standard methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the MuOPG--Met-Lys[22-401] gene.

Expression of recombinant MuOPG Met-Lys[22-401] polypeptide from transformed 393 cells harboring the recombinant pAMG21 plasmid following induction was determined using methods described in Section C.

Oligo #1282-95

5'-TAT GAA AGA AAC TCT GCC TCC AAA ATA CCT GCA TTA CGA TCC GGA AAC TGG TCA TCA GCT GCT GTG TGA TAA ATG TGC TCC GGG TAC-3' (SEQ ID NO:71)

Oligo #1282-96

5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CCG GAT CGT AAT GCA GGT ATT TTG GAG GCA GAG TTT CTT TCA-3' (SEQ ID NO:72)

G. Murine OPG met-lys-(his)$_7$[22-401]

A DNA sequence coding for N-terminal residues Met-Lys-His-His-His-His-His-His-His (=MKH) followed by amino acids 22 through 401 of Murine OPG was placed under control of the lux PR promoter in prokaryotic expression vector pAMG21 as follows. PCR was performed using oligonucleotides #1300-50 and #1257-15 as primers and plasmid pAMG21-muOPG-met[22-401] DNA as template. Thermocycling conditions were as described in Section B. The resulting PCR sample was resolved on an agarose gel, the PCR product was excised, purified, cleaved with NdeI and BamHI restriction endonucleases and purified. The NdeI and BamHI digested and purified PCR product generated using oligo primers #1300-50 and #1257-15 was directionally inserted between the NdeI and BamHI sites of pAMG21 using standard DNA methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing performed to verify the DNA sequence of the muOPG-MKH[22-401] gene.

Expression of recombinant MuOPG-MKH[22-401] polypeptide from transformed 393 cultures harboring the recombinant pAMG21 plasmid following induction was determined using methods described in Section C.

Oligo #1300-50

5'-GTT CTC CTC ATA TGA AAC ATC ATC ACC ATC ACC ATC ATG AAA CTC TGC CTC CAA AAT ACC TGC ATT ACG AT-3' (SEQ ID NO:73)

Oligo #1257-15

(see Section D)

H. Murine OPG met-lys[22-401](his)7

A DNA sequence coding for a N-terminal met-lys, amino acids 22 through 401 murine OPG, and seven histidine residues following amino acid 401 (=muOPG MK[22-401]-H7), was placed under control of the lux PR promoter in prokaryotic expression vector pAMG21 as follows. PCR was performed using oligonucleotides #1300-49 and #1300-51 as primers and pAMG21-muOPG met[22-401] DNA as template. Thermocycling conditions were as described in Section B. The resulting PCR sample was resolved on an agarose gel, the PCR product was excised, purified, restricted with NdeI and BamHI restriction endonucleases, and purified. The NdeI and BamHI digested and purified PCR product was directionally inserted between the NdeI and BamHI sites in pAMG21 using standard methodology. The ligation was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the muOPG MK[22-401]-H7 gene.

Expression of the recombinant muOPG MK-[22-401]-H7 polypeptide from a transformed 393 cells harboring the recombinant pAMG21 plasmid following induction was determined using methods described in Section C.

Oligo #1300-49

5'-GTT CTC CTC ATA TGA AAG AAA CTC TGC CTC CAA AAT ACC TGC A-3' (SEQ ID NO:74)

Oligo #1300-51

5'-TAC GCA CTG GAT CCT TAA TGA TGG TGA TGG TGA TGA TGT AAG CAG CTT ATT TTC ACG GAT TGA ACC TGA TTC CCT A-3' (SEQ ID NO:75)

I. Murine OPG met[27-401]

A DNA sequence coding for a N-terminal methionine and amino acids 27 through 401 of murine OPG was placed under control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. PCR was performed with oligonucleotides #1309-74 and #1257-15 as primers and plasmid pAMG21-muOPG-met[22-401] DNA as template. Thermocycling conditions were as described in Section B. The resulting PCR sample was resolved on an agarose gel, the PCR product was excised, purified, cleaved with NdeI and BamHI restriction endonucleases, and purified. The NdeI and BamHI digested and purified PCR product was directionally inserted between the NdeI and BamHI sites of pAMG21 using standard methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the muOPG-met[27-401] gene.

Expression of recombinant muOPG-met[27-401] polypeptide from a transfected 393 culture harboring the recombinant pAMG21 plasmid following induction was determined using methods described in Section C.

Oligo#1309-74

5'-GTT CTC CTC ATA TGA AAT ACC TGC ATT ACG ATC CGG AAA CTG GTC AT-3' (SEQ ID NO:76)

Oligo#1257-15

(See Section D)

J. Human OPG met[27-401]

A DNA sequence coding for a N-terminal methionine and amino acids 27 through 401 of human OPG was placed under control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. PCR was performed using oligonucleotides #1309-75 and #1309-76 as primers and plasmid pAMG21-huOPG-met[22-401] DNA as template. Thermocycling conditions were as described in Section B. The resulting PCR sample was resolved on an agarose gel, the PCR product was excised, purified, restricted with AseI and BamHI restriction endonucleases, and purified. The AseI and BamHI digested and purified PCR product above was directionally inserted between the NdeI and BamHI sites of pAMG21 using standard methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the huOPG-met[27-401] gene.

Expression of the recombinant huOPG-met[27-401] polypeptide following induction of from transfected 393 cells harboring the recombinant pAMG21 plasmid was determined using methods described in Section C.

Oligo #1309-75

5'-GTT CTC CTA TTA ATG AAA TAT CTT CAT TAT GAT GAA GAA ACT T-3' (SEQ ID NO:77)

Oligo #1309-76

5'-TAC GCA CTG GAT CCT TAT AAG CAG CTT ATT TTT ACT GAT T-3' (SEQ ID NO:78)

K. Murine OPG met[22-180]

A DNA sequence coding for a N-terminal methionine and amino acids 22 through 180 of murine OPG was placed under control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. PCR was performed with oligonucleotides #1309-72 and #1309-73 as primers and plasmid pAMG21-muOPG-met[22-401] DNA as template. Thermocycling conditions were as described in Section B. The resulting PCR sample was resolved on an agarose gel, the PCR product was excised, purified, restricted with NdeI and BamHI restriction endonucleases, and purified. The NdeI and BamHI digested and purified PCR product above was directionally inserted between the NdeI and BamHI sites of pAMG21 using standard methodology. The ligation was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the muOPG-met[22-180] gene.

Expression of recombinant muOPG-met[22-180] polypeptide from transformed 393 cultures harboring the recombinant pAMG21 plasmid following induction was determined using methods described in Section C.

Oligo #1309-72

5'-GTT CTC CTC ATA TGG AAA CTC TGC CTC CAA AAT ACC TGC A-3' (SEQ ID NO:79)

Oligo #1309-73

5'-TAC GCA CTG GAT CCT TAT GTT GCA TTT CCT TTC TGA ATT AGC A-3' (SEQ ID NO:80)

L. Murine OPG met[27-180]

A DNA sequence coding for a N-terminal methionine and amino acids 27 through 180 of murine OPG was placed under the control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. PCR was performed using oligonucleotides #1309-74 (see Section I) and #1309-73 (see Section K) as primers and plasmid pAMG21-muOPG met[22-401] DNA as template. Thermocycling conditions were as described in Section B. The resulting PCR sample was resolved on an agarose gel, the PCR product excised, purified, restricted with NdeI and BamHI restriction endonucleases, and purified. The NdeI and BamHI digested and purified PCR product above was directionally inserted between the NdeI and BamHI sites in pAMG21 using standard methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the muOPG met[27-180] gene.

Expression of recombinant muOPG met[27-180] polypeptide from cultures of transformed 393 cells harboring the recombinant pAMG21 plasmid following induction was determined using methods described in Section C.

M. Murine OPG met[22-189] and met[22-194]

A DNA sequence coding for a N-terminal methionine and either amino acids 22 through 189, or 22 through 194 of murine OPG was placed under control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. The pair of synthetic oligonucleotides #1337-92 and #1337-93 (=muOPG-189 linker) or #1333-57 and #1333-58 (=muOPG-194 linker) were phosphorylated individually and allowed to form an oligo linker duplex pair using methods described in Section B. Purified plasmid DNA of pAMG21-muOPG-met[22-401] was cleaved with KpnI and BspEI restriction endonucleases and the resulting DNA fragments were resolved on an agarose gel. The ~413 bp B fragment was isolated using standard recombinant DNA methodology. The phosphorylated oligo linker duplexes formed between either oligos #1337-92 and #1337-93 (muOPG-189 linker) or oligos #1333-57 and #1333-58 (muOPG-194 linker) containing BspEI and BamHI cohesive ends, and the isolated ~413 bp B fragment of plasmid pAMG21-muOPG-met[22-401] digested with KpnI and BspEI restriction endonucleases above, was directionally inserted between the KpnI and BamHI sites of pAMG21-muOPG met[22-401] using standard methodology. Each ligation mixture was transformed into *E. coli* host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of either the muOPG-met[22-189] or muOPG-met[22-194] genes.

Expression of recombinant muOPG-met[22-189] and muOPG-met[22-194] polypeptides from recombinant pAMG21 plasmids transformed into 393 cells was determined using methods described in Section C.

Oligo #1337-92
5'-CCG GAA ACA GAT AAT GAG-3' (SEQ ID NO:81)

Oligo #1337-93
5'-GAT CCT CAT TAT CTG TTT-3' (SEQ ID NO:82)

Oligo #1333-57
5'-CCG GAA ACA GAG AAG CCA CGC AAA AGT AAG-3' (SEQ ID NO:83)

Oligo #1333-58
5'-GAT CCT TAC TTT TGC GTG GCT TCT CTG TTT-3' (SEQ ID NO:84)

N. Murine OPG met[27-189] and met[27-194]

A DNA sequence coding for a N-terminal methionine and either amino acids 27 through 189, or 27 through 194 of murine OPG was placed under control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. Phosphorylated oligo linkers either "muOPG-189 linker" or "muOPG-194 linker" (see Section M) containing BspEI and BamHI cohesive ends, and the isolated ~413 bp B fragment of plasmid pAMG21-muOPG-met[22-401] digested with KpnI and BspEI restriction endonucleases were directionally inserted between the KpnI and BamHI sites of plasmid pAMG21-muOPG-met[27-401] using standard methodology. Each ligation was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of either the muOPG met[27-189] or muOPG met[27-194] genes.

Expression of recombinant muOPG met[27-189] and muOPG met[27-194] following induction of 393 cells

harboring recombinant pAMG21 plasmids was determined using methods described in Section C.

O. Human OPG met[22-185], met[22-189], met[22-194]

A DNA sequence coding for a N-terminal methionine and either amino acids 22 through 185, 22 through 189, or 22 through 194 of the human OPG polypeptide was placed under control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. The pair of synthetic oligonucleotides #1331-87 and #1331-88 (=huOPG-185 linker), #1331-89 and #1331-90 (=huOPG-189 linker), or #1331-91 & #1331-92 (=huOPG-194 linker) were phosphorylated individually and each allowed to form an oligo linker duplex pair using methods described in Section B. Purified plasmid DNA of pAMG21-huOPG-met[27-401] was restricted with KpnI and NdeI restriction endonucleases and the resulting DNA fragments were resolved on an agarose gel. The ~407 bp B fragment was isolated using standard recombinant DNA methodology. The phophorylated oligo linker duplexes formed between either oligos #1331-87 and #1331-88 (huOPG-185 linker), oligos #1331-89 and #1331-90 (huOPG-189 linker), or oligos #1331-91 and #1331-92 (huOPG-194 linker)[each linker contains NdeI and BamHI cohesive ends], and the isolated ~407 bp B fragment of plasmid pAMG21-huOPG-met[27-401] digested with KpnI and NdeI restriction endonucleases above, was directionally inserted between the KpnI and BamHI sites of plasmid pAMG21-huOPG-met[22-401] using standard methodology. Each ligation was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of

either the huOPG-met[22-185], huOPG-met[22-189], or huOPG-met[22-194] genes.

Expression of recombinant huOPG-met[22-185], huOPG-met[22-189] or huOPG-met[22-194] in transformed 393 cells harboring recombinant pAMG21 plasmids following induction was determined using methods described in Section C.

Oligo #1331-87
5'-TAT GTT AAT GAG-3' (SEQ ID NO:85)

Oligo #1331-88
5'-GAT CCT CAT TAA CA-3' (SEQ ID NO:86)

Oligo #1331-89
5'-TAT GTT CCG GAA ACA GTT AAG-3' (SEQ ID NO:87)

Oligo #1331-90
5'-GAT CCT TAA CTG TTT CCG GAA CA-3' (SEQ ID NO:88)

Oligo #1331-91
5'-TAT GTT CCG GAA ACA GTG AAT CAA CTC AAA AAT AAG-3' (SEQ ID NO:89)

Oligo #1331-92
5'-GAT CCT TAT TTT TGA GTT GAT TCA CTG TTT CCG GAA CA-3' (SEQ ID NO:90)

P. Human OPG met[27-185], met[27-189], met [27-194]

A DNA sequence coding for a N-terminal methionine and either amino acids 27 through 185, 27 through 189, or 27 through 194 of the human OPG polypeptid was placed under control of the lux PR

promoter of prokaryotic expression vector pAMG21 as follows. Phosphorylated oligo linkers "huOPG-185 linker", "huOPG-189 linker", or "huOPG-194 linker" (See Section O) each containing NdeI and BamHI cohesive ends, and the isolated ~407 bp B fragment of plasmid pAMG21-huOPG-met[27-401] digested with KpnI and NdeI restriction endonucleases (See Section O) were directionally inserted between the KpnI and BamHI sites of plasmid pAMG21-huOPG-met[27-401] (See Section J) using standard methodology. Each ligation was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA isolated, and DNA sequencing performed to verify the DNA sequence of either the huOPG-met[27-185], huOPG-met[27-189], or huOPG-met[27-194] genes.

Expression of recombinant huOPG-met[27-185], huOPG-met[27-189], and huOPG-met[27-194] from recombinant pAMG21 plasmids transformed into 393 cells was determined using methods described in Section C.

O. Murine OPG met[27-401] (P33E, G36S, A45P)

A DNA sequence coding for an N-terminal methionine and amino acids 27 through 48 of human OPG followed by amino acid residues 49 through 401 of murine OPG was placed under control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. Purified plasmid DNA of pAMG21-huOPG-met[27-401] (See Section J) was cleaved with AatII and KpnI restriction endonucleases and a ~1075 bp B fragment isolated from an agarose gel using standard recombinant DNA methodology. Additionally, plasmid pAMG21-muOPG-met[22-401] DNA (See Section D) was digested with KpnI and BamHI restriction

endonucleases and the ~1064 bp B fragment isolated as described above. The isolated ~1075 bp pAMG21-huOPG-met[27-401] restriction fragment containing AatII & KpnI cohesive ends (see above), the ~1064 bp pAMG21-muOPG-met[22-401] restriction fragment containing KpnI and BamHI sticky ends and a ~5043 bp restriction fragment containing AatII and BamHI cohesive ends and corresponding to the nucleic acid sequence of pAMG21 between AatII & BamHI were ligated using standard recombinant DNA methodology. The ligation was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, and the presence of the recombinant insert in the plasmid verified using standard DNA methodology. muOPG-27-401 (P33E, G36S, A45P) gene. Amino acid changes in muOPG from proline-33 to glutamic acid-33, glycine-36 to serine-36, and alanine-45 to proline-45, result from replacement of muOPG residues 27 through 48 with huOPG residues 27 through 48.

Expression of recombinant muOPG-met[27-401] (P33E, G36S, A45P) from transformed 393 cells harboring the recombinant pAMG21 plasmid was determined using methods described in Section C.

R. Murine OPG met-lys-(his)$_7$-ala-ser-(asp)$_4$-lys[22-401] (A45T)

A DNA sequence coding for an N-terminal His tag and enterokinase recognition sequence which is (NH$_2$ to COOH terminus): Met-Lys-His-His-His-His-His-His-His-Ala-Ser-Asp-Asp-Asp-Asp-Lys (=HEK), followed by amino acids 22 through 401 of the murine OPG polypeptide was placed under control of the lac r pressor regulated Ps4 promoter as follows. pAMG22-His (See Section A) was

digested with NheI and BamHI restriction endonucleases, and the large fragment (the A fragment) isolated from an agarose gel using standard recombinant DNA methodology. Oligonucleotides #1282-91 and #1282-92 were phosphorylated individually and allowed to form an oligo linker duplex using methods previously described (See Section B). The phosphorylated linker duplex formed between oligos #1282-91 and #1282-92 containing NheI and KpnI cohesive ends, the KpnI and BamHI digested and purified PCR product described (see Section D), and the A fragment of vector pAMG22-His digested with NheI and BamHI were ligated using standard recombinant DNA methodology. The ligation was transformed into E. coli host GM120 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA isolated and DNA sequencing performed to verify the DNA sequence of the muOPG-HEK[22-401] gene. DNA sequencing revealed a spurious mutation in the natural muOPG sequence that resulted in a single amino acid change of Alanine-45 of muOPG polypeptide to a Threonine.

Expression of recombinant muOPG-HEK[22-401] (A45T) from GM120 cells harboring the recombinant pAMG21 plasmid was determined using methods similar to those described in Section C, except instead of addition of the synthetic autoinducer, IPTG was added to 0.4 mM final to achieve induction.

Oligo #1282-91

5'-CTA GCG ACG ACG ACG ACA AAG AAA CTC TGC CTC CAA AAT ACC TGC ATT ACG ATC CGG AAA CTG GTC ATC AGC TGC TGT GTG ATA AAT GTG CTC CGG GTA C-3' (SEQ ID NO:91)

Oligo #1282-92

5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CCG GAT CGT AAT GCA GGT ATT TTG GAG GCA GAG TTT CTT TGT CGT CGT CGT CG-3' (SEQ ID NO:92)

S. Human OPG met-arg-gly-ser-(his)$_6$[22-401]

Eight oligonucleotides (1338-09 to 1338-16 shown below) were designed to produce a 175 base fragment as overlapping, double stranded DNA. The oligos were annealed, ligated, and the 5' and 3' oligos were used as PCR primers to produce large quantities of the 175 base fragment. The final PCR gene products were digested with restriction endonucleases ClaI and KpnI to yield a fragment which replaces the N-terminal 28 codons of human OPG. The ClaI and KpnI digested PCR product was inserted into pAMG21-huOPG [27-401] which had also been cleaved with ClaI and KpnI. Ligated DNA was transformed into competent host cells of E. coli strain 393. Clones were screened for the ability to produce the recombinant protein product and to possess the gene fusion having the correct nucleotide sequence. Protein expression levels were determined from 50 ml shaker flask studies. Whole cell lysate and sonic pellet were analyzed for expression of the construct by Coomassie stained PAGE gels and Western analysis with murine anti-OPG antibody. Expression of huOPG Met-Arg-Gly-Ser-(His)$_6$ [22-401] resulting in the formation of large inclusion bodies and the protein was localized to the insoluble (pellet) fraction.

1338-09

ACA AAC ACA ATC GAT TTG ATA CTA GA (SEQ ID NO:93)

1338-10

TTT GTT TTA ACT AAT TAA AGG AGG AAT AAA ATA TGA GAG GAT CGC ATC AC (SEQ ID NO:94)

1338-11

CAT CAC CAT CAC GAA ACC TTC CCG CCG AAA TAC CTG CAC TAC GAC GAA GA (SEQ ID NO:95)

1338-12

AAC CTC CCA CCA GCT GCT GTG CGA CAA ATG CCC GCC GGG TAC CCA AAC A (SEQ ID NO:96)

1338-13

TGT TTG GGT ACC CGG CGG GCA TTT GT (SEQ ID NO:97)

1338-14

CGC ACA GCA GCT GGT GGG AGG TTT CTT CGT CGT AGT GCA GGT ATT TCG GC (SEQ ID NO:98)

1338-15

GGG AAG GTT TCG TGA TGG TGA TGG TGA TGC GAT CCT CTC ATA TTT TAT T (SEQ ID NO:99)

1338-16

CCT CCT TTA ATT AGT TAA AAC AAA TCT AGT ATC AAA TCG ATT GTG TTT GT (SEQ ID NO:100)

I. Human OPG met-lys[22-401] and met(lys)3[22-401]

To construct the met-lys and met-(lys)3 versions of human OPG[22-401], overlapping oligonucleotides were designed to add the appropriate number of lysine residues. The two oligos for each construct were designed to overlap, allowing two rounds of PCR to produce the final product. The template for

the first PCR reaction was a plasmid DNA preparation containing the human OPG 22-401 gene. The first PCR added the lysine residue(s). The second PCR used the product of the first round and added sequence back to the first restriction site, ClaI.

The final PCR gene products were digested with restriction endonucleases ClaI and KpnI, which replace the N-terminal 28 codons of hu OPG, and then ligated into plasmid pAMG21-hu OPG [27-401] which had been also digested with the two restriction endonucleases. Ligated DNA was transformed into competent host cells of E. coli strain 393. Clones were screened for the ability to produce the recombinant protein product and to possess the gene fusion having the correct nucleotide sequence. Protein expression levels were determined from 50 ml shaker flask studies. Whole cell lysate and sonic pellet were analyzed for expression of the construct by Coomassie stained PAGE gels and Western analysis with murine anti-OPG antibody. Neither construct had a detectable level of protein expression and inclusion bodies were not visible. The DNA sequences were confirmed by DNA sequencing.

Oligonucleotide primers to prepare Met-Lys huOPG[22-401]:
1338-17
ACA AAC ACA ATC GAT TTG ATA CTA GAT TTG TTT TAA CTA ATT AAA GGA GGA ATA AAA TG (SEQ ID NO:101)

1338-18
CTA ATT AAA GGA GGA ATA AAA TGA AAG AAA CTT TTC CTC CAA AAT ATC (SEQ ID NO:102)

1338-20

TGT TTG GGT ACC CGG CGG ACA TTT ATC ACA C (SEQ ID NO:103)

Oligonucleotide primers to prepare Met-(Lys)$_3$-huOPG[22-401]:

1338-17
ACA AAC ACA ATC GAT TTG ATA CTA GAT TTG TTT TAA CTA ATT AAA GGA GGA ATA AAA TG (SEQ ID NO:104)

1338-19
CTA ATT AAA GGA GGA ATA AAA TGA AAA AAA AAG AAA CTT TTC CTC CAA AAT ATC (SEQ ID NO:105)

1338-20
TGT TTG GGT ACC CGG CGG ACA TTT ATC ACA C (SEQ ID NO:106)

U. Human and Murine OPG [22-401]/Fc Fusions

Four OPG-Fc fusions were constructed where the Fc region of human IgG1 was fused at the N-terminus of either human or murine Osteoprotegerin amino acids 22 to 401 (referred to as Fc/OPG [22-401]) or at the C-terminus (referred to as OPG[22-401]/Fc). Fc fusions were constructed using the fusion vector pFc-A3 described in Example 7.

All fusion genes were constructed using standard PCR technology. Template for PCR reactions were plasmid preparations containing the target genes. Overlapping oligos were designed to combine the C-terminal portion of one gene with the N terminal portion of the other gene. This process allows fusing the two genes together in the correct reading frame after the appropriate PCR reactions have be n performed. Initially one "fusion" oligo for each gene was put into

a PCR reaction with a universal primer for the vector carrying the target gene. The complimentary "fusion" oligo was used with a universal primer to PCR the other gene. At the end of this first PCR reaction, two separate products were obtained, with each individual gene having the fusion site present, creating enough overlap to drive the second round of PCR and create the desired fusion. In the second round of PCR, the first two PCR products were combined along with universal primers and via the overlapping regions, the full length fusion DNA sequence was produced.

The final PCR gene products were digested with restriction endonucleases XbaI and BamHI, and then ligated into the vector pAMG21 having been also digested with the two restriction endonucleases. Ligated DNA was transformed into competent host cells of E. coli strain 393. Clones were screened for the ability to produce the recombinant protein product and to possess the gene fusion having the correct nucleotide sequence. Protein expression levels were determined from 50 ml shaker flask studies. Whole cell lysate, sonic pellet, and supernatant were analyzed for expression of the fusion by Coomassie stained PAGE gels and Western analysis with murine anti-OPG antibody.

Fc/huOPG [22-401]

Expression of the Fc/hu OPG [22-401] fusion peptide was detected on a Coomassie stained PAGE gel and on a Western blot. The cells have very large inclusion bodies, and the majority of the product is in the insoluble (pellet) fraction. The following primers were used to construct this OPG-Fc fusion:

1318-48

CAG CCC GGG TAA AAT GGA AAC GTT TCC TCC AAA ATA TCT TCA TT (SEQ ID NO:107)

1318-49

CGT TTC CAT TTT ACC CGG GCT GAG CGA GAG GCT CTT CTG CGT GT (SEQ ID NO:108)

Fc/muOPG [22-401]

Expression of the fusion peptide was detected on a Coomassie stained gel and on a Western blot. The cells have very large inclusion bodies, and the majority of the product is in the insoluble (pellet) fraction. The following primers were used to construct this OPG-Fc fusion:

1318-50

CGC TCA GCC CGG GTA AAA TGG AAA CGT TGC CTC CAA AAT ACC TGC (SEQ ID NO:109)

1318-51

CCA TTT TAC CCG GGC TGA GCG AGA GGC TCT TCT GCG TGT (SEQ ID NO:110)

muOPG [22-401]/Fc

Expression of the fusion peptide was detected on a Coomassie stained gel and on a Western blot. The amount of recombinant product was less than the OPG fusion proteins having the Fc region in the N terminal position. Obvious inclusion bodies were not detected. Most of the product appeared to be in the insoluble (pellet) fraction. The following primers were used to construct this OPG-Fc fusion:

1318-54
GAA AAT AAG CTG CTT AGC TGC AGC TGA ACC AAA ATC
(SEQ ID NO:111)

1318-55
CAG CTG CAG CTA AGC AGC TTA TTT TCA CGG ATT G
(SEQ ID NO:112)

huOPG [22-401]/Fc

Expression of the fusion peptide was not detected on a Coomassie stained gel, although a faint Western positive signal was present. Obvious inclusion bodies were not detected. The following primers were used to prepare this OPG-Fc fusion:

1318-52
AAA AAT AAG CTG CTT AGC TGC AGC TGA ACC AAA ATC
(SEQ ID NO:113)

1318-53
CAG CTG CAG CTA AGC AGC TTA TTT TTA CTG ATT GG
(SEQ ID NO:114)

V. Human OPG met[22-401]-Fc fusion (P25A)

This construct combines a proline to alanine amino acid change at position 25 (P25A) with the huOPG met[22-401]-Fc fusion. The plasmid was digested with restriction endonucleases ClaI and KpnI, which removes the N-terminal 28 codons of the gene, and the resulting small (less than 200 base pair) fragment was gel purified. This fragment containing the proline to alanine change was then ligated into plasmid pAMG21-huOPG [22-401]-Fc fusion which had been digested with

the two restriction endonucleases. The ligated DNA was transformed into competent host cells of *E. coli* strain 393. Clones were screened for the ability to produce the recombinant protein product and to possess the gene fusion having the correct nucleotide sequence. Protein expression levels were determined from 50 ml shaker flask studies. Whole cell lysate and sonic pellet were analyzed for expression of the construct by Coomassie stained PAGE gels and Western analysis with murine anti-OPG antibody. The expression level of the fusion peptide was detected on a Coomassie stained PAGE gel and on a Western blot. The protein was in the insoluble (pellet) fraction. The cells had large inclusion bodies.

W. Human OPG met[22-401] (P25A)

A DNA sequence coding for an N-terminal methionine and amino acids 22 through 401 of human OPG with the proline at position 25 being substituted by alanine under control of the lux P_R promoter in prokaryotic expression vector pAMG21 was constructed as follows: Synthetic oligos # 1289-84 and 1289-85 were annealed to form an oligo linker duplex with XbaI and KpnI cohesive ends. The synthetic linker duplex utilized optimal *E. coli* codons and encoded an N-terminal methionine. The linker also included an SpeI restriction site which was not present in the original sequence. The linker duplex was directionally inserted between the XbaI and KpnI sites in pAMG21-huOPG-22-401 using standard methods. The ligation mixture was introduced into *E. coli* host GM221 by transformation. Clones were initially screened for production of the recombinant protein. Plasmid DNA was isolated from positive clones and DNA sequencing was performed to

verify the DNA sequence of the HuOPG-Met[22-401](P25A) gene. The following oligonucleotides were used to generate the XbaI - KpnI linker:

Oligo #1289-84

5'-CTA GAA GGA GGA ATA ACA TAT GGA AAC TTT TGC TCC AAA ATA TCT TCA TTA TGA TGA AGA AAC TAG TCA TCA GCT GCT GTG TGA TAA ATG TCC GCC GGG TAC -3' (SEQ ID NO:115)

Oligo #1289-85

5'- CCG GCG GAC ATT TAT CAC ACA GCA GCT GAT GAC TAG TTT CTT CAT CAT AAT GAA GAT ATT TTG GAG CAA AAG TTT CCA TAT GTT ATT CCT CCT T-3' (SEQ ID NO:116)

X. Human OPG met[22-401] (P26A) and (P26D)

A DNA sequence coding for an N-terminal methionine and amino acids 22 through 401 of human OPG with the proline at position 26 being substituted by alanine under control of the lux P_R promoter in prokaryotic expression vector pAMG21 was constructed as follows: Synthetic oligos # 1289-86 and 1289-87 were annealed to form an oligo linker duplex with XbaI and SpeI cohesive ends. The synthetic linker duplex utilized optimal E. coli codons and encoded an N-terminal methionine. The linker duplex was directionally inserted between the XbaI and SpeI sites in pAMG21-huOPG[22-401](P25A) using standard methods. The ligation mixture was introduced into E. coli host GM221 by transformation. Clones were initially screened for production of the recombinant protein. Plasmid DNA was isolated from positive clones and DNA sequencing was performed to verify the DNA sequence of the huOPG-m t[22-401](P26A) gene. One of the clones sequenced was

found to have the proline at position 26 substituted by aspartic acid rather than alanine, and this clone was designated huOPG-met[22-401](P26D). The following oligonucleotides were used to generate the XbaI - SpeI linker:

Oligo #1289-86

5' - CTA GAA GGA GGA ATA ACA TAT GGA AAC TTT TCC TGC TAA ATA TCT TCA TTA TGA TGA AGA AA - 3' (SEQ ID NO:117)

Oligo #1289-87

5' - CTA GTT TCT TCA TCA TAA TGA AGA TAT TTA GCA GGA AAA GTT TCC ATA TGT TAT TCC TCC TT - 3' (SEQ ID NO:118)

Y. Human OPG met[22-194] (P25A)

A DNA sequence coding for an N-terminal methionine and amino acids 22 through 194 of human OPG with the proline at position 25 being substituted by alanine under control of the lux P_R promoter in prokaryotic expression vector pAMG21 was constructed as follows: The plasmids pAMG21-huOPG[27-194] and pAMG21-huOPG[22-401] (P25A) were each digested with KpnI and BamHI endonucleases. The 450 bp fragment was isolated from pAMG21-huOPG[27-194] and the 6.1 kbp fragment was isolated from pAMG21-huOPG[22-401] (P25A). These fragments were ligated together and introduced into E. coli host GM221 by transformation. Clones were initially screened for production of the recombinant protein. Plasmid DNA was isolated from positive clones

and DNA sequencing was performed to verify the DNA sequence of the huOPG-Met[22-194](P25A) gene.

EXAMPLE 9

Association of OPG Monomers

CHO cells engineered to overexpress muOPG [22-401] were used to generate conditioned media for the analysis of secreted recombinant OPG using rabbit polyclonal anti-OPG antibodies. An aliquot of conditioned media was concentrated 20-fold, then analysed by reducing and non-reducing SDS-PAGE (Figure 15). Under reducing conditions, the protein migrated as a Mr 50-55 kd polypeptide, as would be predicted if the mature product was glycosylated at one or more of its consensus N-linked glycosylation sites. Suprisingly, when the same samples were analysed by non-reducing SDS-PAGE, the majority of the protein migrated as an approximately 100 kd polypeptide, twice the size of the reduced protein. In addition, there was a smaller amount of the Mr 50-55 kd polypeptide. This pattern of migration on SDS-PAGE was consistent with the notion that the OPG product was forming dimers through oxidation of a free sulfhydryl group(s).

The predicted mature OPG polypeptide contains 23 cysteine residues, 18 of which are predicted to be involved in forming intrachain disulfide bridges which comprise the four cysteine-rich domains (Figure 12A). The five remaining C-terminal cysteine residues are not involved in secondary structure which can be predicted based upon homology with other TNFR family members. Overall there is a net uneven number of cysteine residues, and it is formally possible that at least one

residue is free to form an intermolecular disulfide bond between two OPG monomers.

To help elucidate patterns of OPG kinesis and monomer association, a pulse-chase labelling study was performed. CHO cells expressing muOPG [22-401] were metabolically labelled as described above in serum-free medium containing ^{35}S methionine and cysteine for 30 min. After this period, the media was removed, and replaced with complete medium containing unlabelled methionine and cysteine at levels approximately 2,000-fold excess to the original concentration of radioactive amino acids. At 30 min, 1hr, 2 hr, 4 hr, 6 hr and 12 hr post addition, cultures were harvested by the removal of the conditioned media, and lysates of the conditioned media and adherent monolayers were prepared. The culture media and cell lysates were clarified as described above, and then immunoprecipitated using anti-OPG antibodies as described above. After the immunoprecipitates were washed, they were released by boiling in non-reducing SDS-PAGE buffer then split into two equal halves. To one half, the reducing agent β-mercaptothanol was added to 5% (v/v) final concentration, while the other half was maintained in non-reducing conditions. Both sets of immunoprecipitates were analysed by SDS-PAGE as described above, then processed for autoradiography and exposed to film. The results are shown in Figure 16. The samples analysed by reducing SDS-PAGE are depicted in the bottom two panels. After synthesis, the OPG polypeptide is rapidly processed to a slightly larger polypeptide, which probably represents modification by N-linked glycoslyation. After approximately 1-2 hours, the level of OPG in the cell decreases dramatically, and concomitantly appears in the culture supernatant. This

appears to be the result of the vectoral transport of OPG from the cell into the media over time, consistent with the notion that OPG is a naturally secreted protein. Analysis of the same immunoprecipitates under nonreducing conditions reveals the relationship between the formation of OPG dimers and secretion into the conditioned media (Figure 16, upper panels). In the first 30-60 minutes, OPG monomers are processed in the cell by apparent glycoslylation, followed by dimer formation. Over time, the bulk of OPG monomers are driven into dimers, which subsequently disappear from the cell. Beginning about 60 minutes after synthesis, OPG dimers appear in the conditioned media, and accumulate over the duration of the experiment. Following this period, OPG dimers are formed, which are then secreted into the culture media. OPG monomers persist at a low level inside the cell over time, and small amounts also appear in the media. This does not appear to be the result of breakdown of covalent OPG dimers, but rather the production of sub-stoichiometric amounts of monomers in the cell and subsequent secretion.

Recombinantly produced OPG from transfected CHO cells appears to be predominantly a dimer. To determine if dimerization is a natural process in OPG synthesis, we analysed the conditioned media of a cell line found to naturally express OPG. The CTLL-2 cell line, a murine cytotoxic T lymphocytic cell line (ATCC accession no. TIB-214), was found to express OPG mRNA in a screen of tissue and cell line RNA. The OPG transcript was found to be the same as the cloned and sequenced 2.5-3.0 kb RNA identified from kidney and found to encode a secreted molecule. Western blot analysis of conditioned media obtained from CTLL-2 cells

shows that most, if not all, of the OPG protein secreted is a dimer (Figure 17). This suggests that OPG dimerization and secretion is not an artifact of overexpression in a cell line, but is likely to be the main form of the product as it is produced by expressing cells.

Normal and transgenic mouse tissues and serum were analysed to determine the nature of the OPG molecule expressed in OPG transgenic mice. Since the rat OPG cDNA was expressed under the control of a hepatocyte control element, extracts made from the parenchyma of control and transgenic mice under non-reducing conditions were analysed (Figure 18). In extract from transgenic, but not control mice, OPG dimers are readily detected, along with substoichiometric amounts of monomers. The OPG dimers and monomers appear identical to the recombinant murine protein expressed in the genetically engineered CHO cells. This strongly suggests that OPG dimers are indeed a natural form of the gene product, and are likely to be key active components. Serum samples obtained from control and transgenic mice were similarly analysed by western blot analysis. In control mice, the majority of OPG protein migrates as a dimer, while small amounts of monomer are also detected. In addition, significant amounts of a larger OPG related protein is detected, which migrates with a relative molecular mass consistent with the predicted size of a covalently-linked trimer. Thus, recombinant OPG is expressed predominantly as a dimeric protein in OPG transgenic mice, and the dimer form may be the basis for the osteopetrotic phenotype in OPG mice. OPG recombinant pr tein may also exist in higher m lecular weight "trimeric" forms.

To determine if the five C-terminal cysteine residues of OPG play a role in homodimerization, the murine OPG codons for cytsteine residues 195 (C195), C202, C277, C319, and C400 were changed to serine using the QuickChange™ Site-Directed Mutagenesis Kit (Stratagene, San Diego, CA) as described above. The muOPG gene was subcloned between the Not I and Xba I sites of the pcDNA 3.1 (+) vector (Invitrogen, San Diego, CA). The resulting plasmid, pcDNA3.1-muOPG, and mutagenic primers were treated with Pfu polymerase in the presence of deoxynucleotides, then amplified in a thermocycler as described above. An aliqout of the reaction is then transfected into competent E. coli XL1-Blue by heatshock, then plated. Plasmid DNA from transformants was then sequenced to verify mutations.

The following primer pairs were used to change the codon for cysteine residue 195 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C195S protein:

1389-19:

5' -CAC GCA AAA GTC GGG AAT AGA TGT CAC-3'
(SEQ ID NO:150)

1406-38:

5' -GTG ACA TCT ATT CCC GAC TTT TGC GTG-3'
(SEQ ID NO:151)

The following primer pairs were used to change the codon for cysteine residue 202 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C202S protein:

1389-21:

5' -CAC CCT GTC GGA AGA GGC CTT CTT C-3'
(SEQ ID NO:152)

1389-22:

5' -GAA GAA GGC CTC TTC CGA CAG GGT G-3' (1389-22)
(SEQ ID NO:153)

The following primer pairs were used to change the codon for cysteine residue 277 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C277S protein:

1389-23:

5' -TGA CCT CTC GGA AAG CAG CGT GCA-3'
(SEQ ID NO:154)

1389-24:

5' -TGC ACG CTG CTT TCC GAG AGG TCA-3'
(SEQ ID NO:155)

The following primer pairs were used to change the codon for cysteine residue 319 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C319S protein:

1389-17:

5' -CCT CGA AAT CGA GCG AGC AGC TCC-3'
(SEQ ID NO:156)

1389-18:

5' -CGA TTT CGA GGT CTT TCT CGT TCT C-3'
(SEQ ID NO:157)

The following primer pairs were used to change the codon for cysteine residue 400 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C400S protein:

1406-72:

5′ -CCG TGA AAA TAA GCT CGT TAT AAC TAG GAA TGG-3′ (SEQ ID NO:158)

1406-75:

5′ -CCA TTC CTA GTT ATA ACG AGC TTA TTT TCA CGG-3′ (SEQ ID NO:159)

Each resulting muOPG [22-401] plasmid containing the appropriate mutation was then transfected into human 293 cells, the mutant OPG-Fc fusion protein purified from conditioned media as described above. The biological activity of each protein was assessed the in vitro osteoclast forming assay described in example 11. Conditioned media from each transfectant was analysed by non-reducing SDS-PAGE and western blotting with anti-OPG antibodies.

Mutation of any of the five C-terminal cysteine residues results in the production of predominantly (>90%) monomeric 55 kd OPG molecules. This strongly suggests that the C-terminal cysteine residues together play a role in OPG homodimerization.

C-terminal OPG deletion mutants were constructed to map the region(s) of the OPG C-terminal domain which are important for OPG homodimerization. These OPG mutants were constructed by PCR amplification using primers which introduce premature stop translation

signals in the C-terminal region of murine OPG. The 5′ oligo was designed to the MuOPG start codon (containing a HindIII restriction site) and the 3′ oligonucleotides (containing a stop codon and XhoI site) were designed to truncate the C-terminal region of muOPG ending at either threonine residue 200 (CT 200), proline 212 (CT212), glutamic acid 293 (CT-293), or serine 355 (CT-355).

The following primers were used to construct muOPG [22-200]:

1091-39:

5′ -CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3′ (SEQ ID NO:160)

1391-91:

5′ -CCT CTC TCG AGT CAG GTG ACA TCT ATT CCA CAC TTT TGC GTG GC-3′ (1391-91) (SEQ ID NO:161)

The following primers were used to construct muOPG [22-212]:

1091-39:

5′ -CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3′ (SEQ ID NO:162)

1391-90:

5′ -CCT CTC TCG AGT CAA GGA ACA GCA AAC CTG AAG AAG GC -3′ (SEQ ID NO:163)

The following primers were used to construct muOPG [22-293]:

1091-39:

5' -CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3' (SEQ ID NO:164)

1391-89:

5'- CCT CTC TCG AGT CAC TCT GTG GTG AGG TTC GAG TGG CC-3' (SEQ ID NO:165)

The following primers were used to construct muOPG [22-355]:

1091-39:

5' -CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3' (SEQ ID NO:166)

1391-88:

5' CCT CTC TCG AGT CAG GAT GTT TTC AAG TGC TTG AGG GC-3' (SEQ ID NO:167)

Each resulting muOPG-CT plasmid containing the appropriate truncation was then transfected into human 293 cells, the mutant OPG-Fc fusion protein purified from conditioned media as described above. The biological activity of each protein was assessed the in vitro osteoclast forming assay described in example 11. The conditioned medias were also analysed by non-reducing SDS-PAGE and western blotting using anti-OPG antibodies.

Truncation of the C-terminal region of OPG effects the ability of OPG to form homodimers. CT 355 is predominantly monomeric, although some dimer is formed. CT 293 forms what appears to be equal molar amounts of monomer and dimer, and also high molecular

weight aggregates. However, CT 212 and CT 200 are monomeric.

EXAMPLE 10
Purification of OPG

A. <u>Purification of mammalian OPG-Fc Fusion Proteins</u>

5 L of conditioned media from 293 cells expressing an OPG-Fc fusion protein were prepared as follows. A frozen sample of cells was thawed into 10 ml of 293S media (DMEM-high glucose, 1x L-glutamine, 10% heat inactivated fetal bovine serum (FBS) and 100 ug/ml hygromycin) and fed with fresh media after one day. After three days, cells were split into two T175 flasks at 1:10 and 1:20 dilutions. Two additional 1:10 splits were done to scale up to 200 T175 flasks. Cells were at 5 days post-thawing at this point. Cells were grown to near confluency (about three days) at which time serum-containing media was aspirated, cells were washed one time with 25 ml PBS per flask and 25 ml of SF media (DMEM-high glucose, 1x L-glutamine) was added to each flask. Cells were maintained at 5% CO2 for three days at which point the media was harvested, centrifuged, and filtered through 0.45m cellulose nitrate filters (Corning).

OPG-Fc fusion proteins were purified using a Protein G Sepharose column (Pharmacia) equilibrated in PBS. The column size varied depending on volume of starting media. Conditioned media prepared as described above was loaded onto the column, the column washed with PBS, and pure protein eluted using 100mM glycine pH 2.7. Fractions were collected into tubes containing 1M Tris pH 9.2 in order to neutralize as quickly as possible. Protein containing fractions were pooled, concentrated

in either an Amicon Centricon 10 or Centriprep 10 and diafiltered into PBS. The pure protein is stored at -80°C.

Murine [22-401]-Fc, Murine [22-180]-Fc, Murine [22-194]-Fc, human [22-401]-Fc and human [22-201]Fc were purified by this procedure. Murine [22-185]-Fc is purified by this procedure.

B. Preparation of anti-OPG antibodies

Three New Zealand White rabbits (5-8 lbs initial wt) were injected subcutaneously with muOPG[22-401]-Fc fusion protein. Each rabbit was immunized on day 1 with 50 μg of antigen emulsified in an equal volume of Freunds complete adjuvant. Further boosts (Days 14 and 28) were performed by the same procedure with the substitution of Freunds incomplete adjuvant. Antibody titers were monitored by EIA. After the second boost, the antisera revealed high antibody titers and 25ml production bleeds were obtained from each animal. The sera was first passed over an affinity column to which murine OPG-Fc had be immobilized. The anti-OPG antibodies were eluted with Pierce Gentle Elution Buffer containing 1% glacial acetic acid. The eluted protein was then dialyzed into PBS and passed over a Fc column to remove any antibodies specific for the Fc portion of the OPG fusion protein. The run through fractions containing anti-OPG specific antibodies were dialyzed into PBS.

C. Purification of murine OPG[22-401]

Antibody Affinity Chromatography

Affinity purified anti-OPG antibodies were diafiltered into coupling buffer (0.1M sodium carbonate

pH 8.3, 0.5M NaCl), and mixed with CNBr-activated sepharose beads (Pharmacia) for two hours at room temperature. The resin was then washed with coupling buffer extensively before blocking unoccupied sited with 1M ethanolamine (pH 8.0) for two hours at room temperature. The resin was then washed with low pH (0.1M sodium acetate pH 4.0, 0.5M NaCl) followed by a high pH wash (0.1M Tris-HCl pH 8.0, 0.5M NaCl). The last washes were repeated three times. The resin was finally equilibrated with PBS before packing into a column. Once packed, the resin was washed with PBS. A blank elution was performed with 0.1M glycine-HCl, pH 2.5), followed by re-equilibration with PBS.

Concentrated conditioned media from CHO cells expressing muOPG[22-410] was applied to the column at a low flow rate. The column was washed with PBS until UV absorbance measured at 280nm returned to baseline. The protein was eluted from the column first with 0.1M glycine-HCl (pH 2.5), re-equilibrated with PBS, and eluted with a second buffer (0.1M CAPS, pH 10.5), 1M NaCl). The two elution pools were diafiltered separately into PBS and sterile filtered before freezing at -20°C.

Conventional Chromatography

CHO cell conditioned media was concentrated 23x in an Amicon spiral wound cartridge (S10Y10) and diafiltered into 20mM tris pH 8.0. The diafiltered media was then applied to a Q-sepharose HP (Pharmacia) column which had been equilibrated with 20mM tris pH 8.0. The column was then washed until absorbence at 280nm reached baseline. Protein was eluted with a 20 column volume gradient of 0-300mM NaCl in tris pH 8.0. OPG protein was detected using a western blot of column fractions.

Fractions containing OPG were pooled and brought to a final concentration of 300mM NaCl, 0.2mM DTT. A NiNTA superose (Qiagen) column was equilibrated with 20mM tris pH 8.0, 300mM NaCl, 0.2mM DTT after which the pooled fractions were applied. The column was washed with equilibration buffer until baseline absorbence was reached. Proteins were eluted from the column with a 0-30mM Imidazole gradient in equilibration buffer. Remaining proteins were washed off the column with 1M Imidazole. Again a western blot was used to detect OPG containing fractions.

Pooled fractions from the NiNTA column were dialyzed into 10mm potassium phosphate pH 7.0, 0.2mM DTT. The dialyzed pool was then applied to a ceramic hydroxyapatite column (Bio-Rad) which had been equilibrated in 10mM phosphate buffer. After column washing, the protein was eluted with a 10-100mM potassium phosphate gradient over 20 column volumes. This was then followed by a 20 column volume gradient of 100-400 mM phosphate.

OPG was detected by coomassie blue staining of SDS-polyacrylamide gels and by western blotting. Fractions were pooled and diafiltered onto PBS and frozen at -80°C. The purified protein runs as a monomer and will remain so after diafiltration into PBS. The monomer is stable when stored frozen or at pH 5 at 4°C. However if stored at 4°C in PBS, dimers and what appears to be trimers and tetramers will form after one week.

D. Purification of human OPG met[22-401] from *E. coli*

The bacterial cell paste was suspended into 10 mM EDTA to a concentration of 15% (w/v) using a low shear homogenizer at 5°C. The cells were then disrupted

by two homogenizations at 15,000 psi each at 5°C. The resulting homogenate was centrifuged at 5,000 x g for one hour at 5°C. The centrifugal pellet was washed by low shear homogenization into water at the original homogenization volume followed by centrifugation as before. The washed pellet was then solubilized to 15% (w/v) by a solution of (final concentration) 6 M guanidine HCl, 10 mM dithiothreitol, 10 mM TrisHCl, pH 8.5 at ambient temperature for 30 minutes. This solution was diluted 30-fold into 2M urea containing 50 mM CAPS, pH 10.5, 1 mM reduced glutathione and then stirred for 72 hours at 5°C. The OPG was purified from this solution at 25°C by first adjustment to pH 4.5 with acetic acid and then chromatography over a column of SP-HP Sepharose resin equilibrated with 25 mM sodium acetate, pH 4.5. The column elution was carried out with a linear sodium chloride gradient from 50 mM to 550 mM in the same buffer using 20 column volumes at a flow rate of 0.1 column volumes/minute. The peak fractions containing only the desired OPG form were pooled and stored at 5°C or buffer exchanged into phosphate buffered saline, concentrated by ultrafiltration, and then stored at 5°C. This material was analyzed by reverse phase HPLC, SDS-PAGE, limulus amebocyte lysate assay for the presence of endotoxin, and N-terminal sequencing. In addition, techniques such as mass spectrometry, pH/temperature stability, fluoresence, circular dichroism, differential scanning calorimetry, and protease profiling assays may also be used to examine the folded nature of the protein.

EXAMPLE 11

Biological Activity of Recombinant OPG

Based on histology and histomorphometry, it appeared that hepatic overexpression of OPG in transgenic mice markedly decreased the numbers of osteoclasts leading to a marked increase in bone tissue (see Example 4). To gain further insight into potential mechanism(s) underlying this *in vivo* effect, various forms of recombinant OPG have been tested in an *in vitro* culture model of osteoclast formation (osteoclast forming assay). This culture system was originally devised by Udagawa (Udagawa et al. Endocrinology 125, 1805-1813 (1989), Proc. Natl. Acad. Sci. USA 87, 7260-7264 (1990)) and employs a combination of bone marrow cells and cells from bone marrow stromal cell lines. A description of the modification of this culture system used for these studies has been previously published (Lacey et al. Endocrinology 136, 2367-2376 (1995)). In this method, bone marrow cells, flushed from the femurs and tibiae of mice, are cultured overnight in culture media (alpha MEM with 10% heat inactivated fetal bovine serum) supplemented with 500 U/ml CSF-1 (colony stimulating factor 1, also called M-CSF), a hematopoietic growth factor specific for cells of the monocyte/macrophage family lineage. Following this incubation, the non-adherent cells are collected, subjected to gradient purification, and then cocultured with cells from the bone marrow cell line ST2 (1×10^6 non-adherent cells : 1×10^5 ST2 cells/ ml media). The media is supplemented with dexamethasone (100 nM) and the biologically-active metabolite of vitamin D3 known as 1,25 dihydroxyvitamin D3 (1,25 (OH)2 D3, 10 nM). To enhance osteoclast appearance, prostaglandin E2 (250 nM)

is added to some cultures. The coculture period usually ranges from 8 - 10 days and the media, with all of the supplements freshly added, is renewed every 3-4 days. At various intervals, the cultures are assessed for the presence of tartrate acid phosphatase (TRAP) using either a histochemical stain (Sigma Kit # 387A, Sigma, St. Louis, MO) or TRAP solution assay. The TRAP histochemical method allows for the identification of osteoclasts phenotypically which are multinucleated (≥ 3 nuclei) cells that are also TRAP+. The solution assay involves lysing the osteoclast-containing cultures in a citrate buffer (100 mM, pH 5.0) containing 0.1% Triton X-100. Tartrate resistant acid phosphatase activity is then measured based on the conversion of p-nitrophenylphosphate (20 nM) to p-nitrophenol in the presence of 80 mM sodium tartrate which occurs during a 3-5 minute incubation at RT. The reaction is terminated by the addition of NaOH to a final concentration of 0.5 M. The optical density at 405 nm is measured and the results are plotted.

Previous studies (Udagawa et al. ibid) using the osteoclast forming assay have demonstrated that these cells express receptors for ^{125}I-calcitonin (autoradiography) and can make pits on bone surfaces, which when combined with TRAP positivity confirm that the multinucleated cells have an osteoclast phenotype. Additional evidence in support of the osteoclast phenotype of the multinucleated cells that arise *in vitro* in the osteoclast forming assay are that the cells express αv and β3 integrins by immunocytochemistry and calcitonin receptor and TRAP mRNA by in situ hybridization (ISH).

The huOPG [22-401]-Fc fusion was purified from CHO cell conditioned media and subsequently utilized in

the osteoclast forming assay. At 100 ng/ml of huOPG [22-401]-Fc, osteoclast formation was virtually 100% inhibited (Figure 19A). The levels of TRAP measured in lysed cultures in microtitre plate wells were also inhibited in the presence of OPG with an ID_{50} of approximately 3 ng/ml (Figure 20). The level of TRAP activity in lysates appeared to correlate with the relative number of osteoclasts seen by TRAP cytochemistry (compare Figures 19A-19G and 20). Purified human IgG1 and TNFbp were also tested in this model and were found to have no inhibitory or stimulatory effects suggesting that the inhibitory effects of the huOPG [22-401]-Fc were due to the OPG portion of the fusion protein. Additional forms of the human and murine molecules have been tested and the cumulative data are summarized in Table 1.

Table 1

Effects of various OPG forms on in vitro osteoclast formation

OPG Construct	Relative Bioactivity in vitro
muOPG [22-401]-Fc	+++
muOPG [22-194]-Fc	+++
muOPG [22-185]-Fc	++
muOPG [22-180]-Fc	-
muOPG [22-401]	+++
muOPG [22-401] C195	+++
muOPG [22-401] C202	+
muOPG [22-401] C277	-
muOPG [22-401] C319	+

muOPG [22-401] C400	+
muOPG [22-185]	-
muOPG [22-194]	++
muOPG [22-200]	++
muOPG [22-212]	-
muOPG [22-293]	+++
muOPG [22-355]	+++
huOPG [22-401]-Fc	+++
huOPG [22-201]-Fc	+++
huOPG [22-401]-Fc P26A	+++
huOPG [22-401]-Fc Y28F	+++
huOPG [22-401]	+++
huOPG [27-401]-Fc	++
huOPG [29-401]-Fc	++
huOPG [32-401]-Fc	+/-

+++, ED_{50} = 0.4-2 ng/ml
++, ED_{50} = 2-10 ng/ml
+, ED_{50} = 10-100 ng/ml
-, ED_{50} > 100 ng/ml

The cumulative data suggest that murine and human OPG amino acid sequences 22-401 are fully active *in vitro*, when either fused to the Fc domain, or unfused. They inhibit in a dose-dependent manner and possess half-maximal activities in the 2-10 ng/ml range. Truncation of the murine C-terminus at threonine residue 180 inactivates the molecule, whereas truncations at cysteine 185 and beyond have full activity. The cysteine residue located at position 185 is predicted to form an SS3 bond in the domain 4 region of OPG. Removal of this residue in other TNFR-related proteins has previously been shown to abrogate biological activity

(Yan et al. J. Biol. Chem. 266, 12099-12104 (1994)). Our finding that muOPG[22-180]-Fc is inactive while muOPG[22-185]-Fc is active is consistent with these findings. This suggests that amino acid residues 22-185 define a region for OPG activity.

These findings indicate that like transgenically-expressed OPG, recombinant OPG protein also suppressed osteoclast formation as tested in the osteoclast forming assay. Time course experiments examining the appearance of TRAP+ cells, β3+ cells, F480+ cells in cultures continuously exposed to OPG demonstrate that OPG blocks the appearance TRAP+ and β3+ cells, but not F480+ cells. In contrast, TRAP+ and β3+ cells begin to appear as early as day 4 following culture establishment in control cultures. Only F480+ cells can be found in OPG-treated cultures and they appear to be present at qualitatively the same numbers as the control cultures. Thus, the mechanism of OPG effects in vitro appears to involve a blockade in osteoclast differentiation at a step beyond the appearance of monocyte-macrophages but before the appearance of cells expressing either TRAP or β3 integrins. Collectively these findings indicate that OPG does not interfere with the general growth and differentiation of monocyte-macrophage precursors from bone marrow, but rather suggests that OPG specifically blocks the selective differentiation of osteoclasts from monocyte-macrophage precursors.

To determine more specifically when in the osteoclast differentiation pathway that OPG was inhibitory, a variation of the in vitro culture method was employed. This variation, described in (Lacey et al. supra), employs bone marrow macrophag s as osteoclast precursors. The osteoclast precursors are

derived by taking the nonadherent bone marrow cells after an overnight incubation in CSF-1/M-CSF, and culturing the cells for an additional 4 days with 1,000 - 2,000 U/ml CSF-1. Following 4 days of culture, termed the growth phase, the non-adherent cells are removed. The adherent cells, which are bone marrow macrophages, can then be exposed for up to 2 days to various treatments in the presence of 1,000 - 2,000 U/ml CSF-1. This 2 day period is called the intermediate differentiation period. Thereafter, the cell layers are again rinsed and then ST-2 cells (1 X 10^5 cell/ml), dexamethasone (100 nM) and 1,25 (OH)2 D3 (10 nM) are added for the last 8 days for what is termed the terminal differentiation period. Test agents can be added during this terminal period as well. Acquisition of phenotypic markers of osteoclast differentiation are acquired during this terminal period (Lacey et al. ibid).

huOPG [22-401]-Fc (100 ng/ml) was tested for its effects on osteoclast formation in this model by adding it during either the intermediate, terminal or, alternatively, both differentiation periods. Both TRAP cytochemistry and solution assays were performed. The results of the solution assay are shown in Figure 21. HuOPG [22-401]-Fc inhibited the appearance of TRAP activity when added to both the intermediate and terminal or only the terminal differentiation phases. When added to the intermediate phase and then removed from the cultures by rinsing, huOPG [22-401]-Fc did not block the appearance of TRAP activity in culture lysates. The cytochemistry results parallel the solution assay data. Collectively, these observations indicate that huOPG [22-401]-Fc only needs to be present during the terminal differentiation period for it to

exert its all of its suppressive effects on osteoclast formation.

B. In vivo IL1-α and IL1-β challenge experiments

IL1 increases bone resorption both systemically and locally when injected subcutaneously over the calvaria of mice (Boyce et al., Endocrinology 125, 1142-1150 (1989)). The systemic effects can be assessed by the degree of hypercalcemia and the local effects histologically by assessing the relative magnitude of the osteoclast-mediated response. The aim of these experiments was to determine if recombinant muOPG [22-401]-Fc could modify the local and/or systemic actions of IL1 when injected subcutaneously over the same region of the calvaria as IL1.

IL-1 β experiment

Male mice (ICR Swiss white) aged 4 weeks were divided into the following treatment groups (5 mice per group): Control group: IL1 treated animals (mice received 1 injection/day of 2.5 ug of IL1-β); Low dose muOPG [22-401]-Fc treated animals (mice received 3 injections/day of 1 μg of muOPG [22-401]-Fc); Low dose muopg [22-401]-Fc and IL1-β; High dose muOPG [22-401]-Fc treated animals (mice receive 3 injections/day of 10 μg muOPG [22-401]-Fc); High dose muOPG [22-401]-Fc and IL1-β. All mice received the same total number of injections of either active factor or vehicle (0.1% bovine serum albumin in phosphate buffered saline). All groups are sacrificed on the day after the last injection. The weights and blood ionized calcium levels are measured before the first injections, four hours after the second injection and 24 hours after the third IL1 injection, just before the animals were sacrificed.

After sacrifice the calvaria were removed and processed for paraffin sectioning.

IL1-α experiment

Male mice (ICR Swiss white) aged 4 weeks were divided into the following treatment groups (5 mice per group): Control group; IL1 alpha treated animals (mice received 1 injection/day of 5 ug of IL1-alpha); Low dose muOPG [22-401]-Fc treated animals (mice received 1 injection/day of 10 μg of muOPG [22-401]-Fc; Low dose muopg [22-401]-Fc and IL1-alpha, (dosing as above); High dose muopg [22-401]-Fc treated animals (mice received 3 injections/day of 10 μg muOPG [22-401]-Fc; High dose muOPG [22-401]-Fc and IL1-α. All mice received the same number of injections/day of either active factor or vehicle. All groups were sacrificed on the day after the last injection. The blood ionized calcium levels were measured before the first injection, four hours after the second injection and 24 hours after the third IL1 injection, just before the animals were sacrificed. The animal weights were measured before the first injection, four hours after the second injection and 24 hours after the third IL1 injection, just before the animals were sacrificed. After sacrifice the calvaria were removed and processed for paraffin sectioning.

Histological methods

Calvarial bone samples were fixed in zinc formalin, decalcified in formic acid, dehydrated through ethanol and mounted in paraffin. Sections (5μm thick) were cut through the calvaria adjacent to the lambdoid suture and stained with either hematoxylin and eosin or reacted for tartrate resistant acid phosphatase activity

(Sigma Kit# 387A) and counterstained with hematoxylin. Bone resorption was assessed in the IL1-α treated mice by histomorphometric methods using the Osteomeasure (Osteometrics, Atlanta, GA) by tracing histologic features onto a digitizor platen using a microscope-mounted camera lucida attachment. Osteoclast numbers, osteoclast lined surfaces, and eroded surfaces were determined in the marrow spaces of the calvarial bone. The injected and non-injected sides of the calvaria were measured separately.

Results

IL1-α and IL1-β produced hypercalcemia at the doses used, particularly on the second day, presumably by the induction of increased bone resorption systemically. The hypercalcemic response was blocked by muOPG [22-401]-Fc in the IL1-beta treated mice and significantly diminished in mice treated with IL1-alpha, an effect most apparent on day 2 (Figure 22A-22B).

Histologic analysis of the calvariae of mice treated with IL1-alpha and beta shows that IL1 treatments alone produce a marked increase in the indices of bone resorption including: osteoclast number, osteoclast lined surface, and eroded surface (surfaces showing deep scalloping due to osteoclastic action (Figure 23B, Table 2). In response to IL1-α or IL1-β, the increases in bone resorption were similar on the injected and non-injected sides of the calvaria. Muopg [22-401]-Fc injections reduced bone resorption in both IL1-alpha and beta treated mice and in mice receiving vehicle alone but this reduction was seen only on the muopg [22-401]-Fc injected sides of the calvariae.

The most likely explanation for these observations is that muOPG [22-401]-Fc inhibited bone

resorption, a conclusion supported by the reduction of both the total osteoclast number and the percentage of available bone surface undergoing bone resorption, in the region of the calvaria adjacent to the muOPG [22-401]-Fc injection sites. The actions of muOPG [22-401]-Fc appeared to be most marked locally by histology, but the fact that muOPG [22-401]-Fc also blunted IL1-induced hypercalcemia suggests that muOPG [22-401]-Fc has more subtle effects on bone resorption systemically.

Table 2. Effects of OPG on variables of bone resorption in IL-1 injected mice.

	Osteoclast Surface % Bone Surface (mean ± S.D)		Eroded Surface %Bone Surface (mean ± S.D)		Osteoclast Number/mm^2 Tissue Area (mean ± S.D)	
Experiment 1	Non-injected side	Injected side	Non-injected side	Injected side	Non-injected side	Injected side
Control	12.36 ± 3.44	9.54 ± 2.46	8.07 ± 3.90	9.75 ± 3.16	32.51 ± 11.09	23.50 ± 10.83
IL1-β (2.5μg/d)	17.18 ± 1.30	16.40 ± 2.16	40.66 ± 4.28	37.53 ± 10.28	71.80 ± 18.76	60.89 ± 5.16
OPG (40μg/d)	10.12 ± 3.71	5.04 ± 1.66	9.73 ± 4.33	4.19 ± 3.61	32.73 ± 11.09	15.24 ± 7.54
OPG+IL1-β	18.61 ± 2.46	# 13.26 ± 2.50	44.87 ± 8.63	# 25.94 ± 6.82	69.42 ± 36.29	# 47.13 ± 24.26
Experiment 2						
Control	11.56 ± 4.22	11.95 ± 2.97	12.67 ± 5.04	10.03 ± 5.13	51.72 ± 23.93	56.03 ± 30.70
IL1-α (5μg/d)	28.81 ± 4.84	23.46 ± 5.76	37.51 ± 5.16	41.10 ± 12.53	113.60 ± 18.04	102.70 ± 32.09
OPG (40μg/d)	14.40 ± 1.00	# 4.26 ± 2.54	11.55 ± 4.14	# 4.29 ± 3.16	72.28 ± 14.11	# 22.65 ± 16.68
OPG+IL1-α	29.58 ± 8.80	# 17.83 ± 3.34	33.66 ± 9.21	# 24.38 ± 8.88	146.10 ± 42.37	# 66.56 ± 15.62

Different to non-injected side $p < 0.05$ (by paired t test)

C. Systemic Effects of muOPG [22-401]-Fc in Growing Mice

Male BDF1 mice aged 3-4 weeks, weight range 9.2- 15.7g were divided into groups of ten mice per group. These mice were injected subcutaneously with saline or muOPG [22-401]-Fc 2.5mg/kg *bid* for 14 days (5mg/kg/day). The mice were radiographed before treatment, at day 7 and on day 14. The mice were sacrificed 24 hours after the final injection. The right femur was removed, fixed in zinc formalin, decalcified in formic acid and embedded in paraffin. Sections were cut through the mid region of the distal femoral metaphysis and the femoral shaft. Bone density, by histomorphometry, was determined in six adjacent regions extending from the metaphyseal limit of the growth plate, through the primary and secondary spongiosa and into the femoral diaphysis (shaft). Each region was 0.5 X 0.5 mm^2.

Radiographic changes

After seven days of treatment there was evidence of a zone of increased bone density in the spongiosa associated with the growth plates in the OPG treated mice relative to that seen in the controls. The effects were particularly striking in the distal femoral and the proximal tibial metaphases (Figure 24A-24B). However bands of increased density were also apparent in the vertebral bodies, the iliac crest and the distal tibia. At 14 days, the regions of opacity had extended further into the femoral and tibial shafts though the intensity of the radio-opacity was diminished. Additionally, there were no differences in the length of the femurs at the completion of the experiment or in the change in length over the duration of the experiment implying that OPG does not alter bone growth.

Histological Changes

The distal femoral metaphysis showed increased bone density in a regions 1.1 to 2.65 mm in distance from the growth plate (Figures 25 and 26A-26B). This is a region where bone is rapidly removed by osteoclast-mediated bone resorption in mice. In these rapidly growing young mice, the increase in bone in this region observed with OPG treatment is consistent with an inhibition of bone resorption.

D. Effects of Osteoprotegerin on Bone Loss Induced by Ovariectomy in the Rat

Twelve week old female Fisher rats were ovariectomized (OVX) or sham operated and dual xray absorptiometry (DEXA) measurements made of the bone density in the distal femoral metaphysis. After 3 days recovery period, the animals received daily injections for 14 days as follows: Ten sham operated animals received vehicle (phosphate buffered saline); Ten OVX animals received vehicle (phosphate buffered saline); Six OVX animals received OPG-Fc 5mg/kg SC; Six OVX animals received pamidronate (PAM) 5mg/kg SC; Six OVX animals received estrogen (ESTR) 40ug/kg SC. After 7 and 14 days treatment the animals had bone density measured by DEXA. Two days after the last injection the animals were killed and the right tibia and femur removed for histological evaluation.

The DEXA measurements of bone density showed a trend to reduction in the bone density following ovariectomy that was blocked by OPG-Fc. Its effects were similar to the known antiresorptive agents estrogen and pamidronate. (Figure 27). The histomorphometric

analysis confirmed these observations with OPG-Fc treatment producing a bone density that was significantly higher in OVX rats than that seen in untreated OVX rats (Figure 28). These results confirm the activity of OPG in the bone loss associated with withdrawal of endogenous estrogen following ovariectomy.

<u>In vivo Summary</u>

The in vivo actions of recombinant OPG parallel the changes seen in OPG transgenic mice. The reduction in osteoclast number seen in the OPG transgenic is reproduced by injecting recombinant OPG locally over the calvaria in both normal mice and in mice treated with IL1-α or IL1-β. The OPG transgenic mice develop an osteopetrotic phenotype with progressive filling of the marrow cavity with bone and unremodelled cartilage extending from the growth plates from day 1 onward after birth. In normal three week old (growing) mice, OPG treatments also led to retention of bone and unremodelled cartilage in regions of endochondral bone formation, an effect observed radiographically and confirmed histologically. Thus, recombinant OPG produces phenotypic changes in normal animals similar to those seen in the transgenic animals and the changes are consistent with OPG-induced inhibition of bone resorption. Based on <u>in vitro</u> assays of osteoclast formation, a significant portion of this inhibition is due to impaired osteoclast formation. Consistent with this hypothesis, OPG blocks ovariectomy-induced osteoporosis in rat. Bone loss in this model is known to be mediated by activated osteoclasts, suggesting a role for OPG in treatment of primary osteoporosis.

EXAMPLE 12

Pegylation Derivatives of OPG

Preparation of N-terminal PEG-OPG conjugates by reductive alkylation

HuOPG met [22-194] P25A was buffer exchanged into 25-50 mM NaOAc, pH 4.5-4.8 and concentrated to 2-5 mg/ml. This solution was used to conduct OPG reductive alkylation with monofunctional PEG aldehydes at 5-7 °C. PEG monofunctional aldehydes, linear or branched, MW=1 to 57 kDa (available from Shearwater Polymers) were added to the OPG solution as solids in amounts constituting 2-4 moles of PEG aldehyde per mole of OPG. After dissolution of polymer into the protein solution, sodium cyanoborohydride was added to give a final concentration of 15 to 20 mM in the reaction mixture from 1-1.6 M freshly prepared stock solution in cold DI water. The progress of the reaction and the extent of OPG PEGylation was monitored by size exclusion HPLC on a G3000SW$_{XL}$ column (Toso Haas) eluting with 100 mM $NaPO_4$, 0.5 M NaCl, 10% ethanol, pH 6.9. Typically the reaction was allowed to proceed for 16-18 hours, after which the reaction mixture was diluted 6-8 times and the pH lowered to 3.5-4. The reaction mixture was fractionated by ion exchange chromatography (HP SP HiLoad 16/10, Pharmacia) eluting with 20 mM NaOAc pH 4 with a linear gradient to 0.75M NaCl over 25 column volumes at a flow rate of 30 cm/h. Fractions of mono-, di- or poly-PEGylated OPG were pooled and characterized by SEC HPLC and SDS-PAGE. By N-terminal sequencing, it was determined that the monoPEG-OPG conjugate, the major reaction product in most cases, was 98% N-terminally PEG-modified OPG.

This procedure was generally used to prepare the following N-terminal PEG-OPG conjugates (where OPG is HuOPG met [22-194] P25A: 5 kD monoPEG, 10 kD mono branched PEG, 12 kD monoPEG, 20 kD monoPEG, 20 kD mono branched PEG, 25 kD monoPEG, 31 kD monoPEG, 57 kD monoPEG, 12 kD diPEG, 25 kD diPEG, 31 kD diPEG, 57 kD diPEG, 25 kD triPEG.

Preparation of PEG-OPG conjugates by acylation

HuOPG met [22-194] P25A was buffer exchanged into 50 mM BICINE buffer, pH 8 and concentrated to 2-3 mg/ml. This solution was used to conduct OPG acylation with monofunctional PEG N-hydroxysuccinimidyl esters at room temperature. PEG N-hydroxysuccinimidyl esters, linear or branched, MW=1 to 57 kDa (available from Shearwater Polymers) were added to the OPG solution as solids in amounts constituting 4-8 moles of PEG N-hydroxysuccinimidyl ester per mole of OPG. The progress of the reaction and the extent of OPG PEGylation was monitored by size exclusion HPLC on a G3000SW$_{XL}$ column (Toso Haas) eluting with 100 mM $NaPO_4$, 0.5 M NaCl, 10% ethanol, pH 6.9. Typically the reaction was allowed to proceed for 1 hour, after which the reaction mixture was diluted 6-8 times and the pH lowered to 3.5-4. The reaction mixture was fractionated by ion exchange chromatography (HP SP HiLoad 16/10, Pharmacia) eluting with 20 mM NaOAc pH 4 with a linear gradient to 0.75M NaCl over 25 column volumes at a flow rate of 30 cm/h. Fractions of mono-, di- or poly- PEGylated OPG were pooled and characterized by SEC HPLC and SDS-PAGE.

This procedure was generally used to prepare the following PEG-OPG conjugates: 5 kD polyPEG, 20 kD polyPEG, 40 kD poly branched PEG, 50 kD poly PEG.

Preparation of dimeric PEG-OPG

HuOPG met [22-194] P25A is prepared for thiolation at 1-3 mg/ml in a phosphate buffer at near neutral pH. S-acetyl mecaptosuccinic anhydride (AMSA) is added in a 3-7 fold molar excess while maintaining pH at 7.0 and the rxn stirred at 4°C for 2 hrs. The monothiolated-OPG is separated from unmodified and polythiolated OPG by ion exchange chromatography and the protected thiol deprotected by treatment with hydroxylamine. After deprotection, the hydroxylamine is removed by gel filtration and the resultant monothiolated-OPG is subjected to a variety of thiol specific crosslinking chemistries. To generate a disulfide bonded dimer, the thiolated OPG at >1mg/ml is allowed to undergo air oxidation by dialysis in slightly basic phosphate buffer. The covalent thioether OPG dimer was prepared by reacting the bis-maleimide crosslinker, N,N-bis(3-maleimido propianyl)-2-hydroxy 1,3 propane with the thiolated OPG at >1mg/ml at a 0.6x molar ratio of crosslinker:OPG in phosphate buffer at pH 6.5. Similarly, the PEG dumbbells are produced by reaction of substoichiometric amounts of bis-maleimide PEG crosslinkers with thiolated OPG at >1mg/ml in phosphate buffer at pH 6.5. Any of the above dimeric conjugates may be further purified using either ion exchange or size exclusion chromatographies.

Dimeric PEG-OPG conjugates (where OPG is HuOPG met [22-194] P25A prepared using the above procedures include disulfide-bonded OPG dimer, covalent thioether OPG dimer with an aliphatic amine type crosslinker, 3.4 kD and 8kD PEG dumbbells and monobells.

PEG-OPG conjugates were tested for activity in vitro using the osteoclast maturation assay described in Example 11A and for activity in vivo by measuring increased bone density after injection into mice as described in Example 11C. The in vivo activity is shown below in Table 3.

Table 3

In vivo biological activity of Pegylated OPG

OPG Construct	Increase in Tibial Bone Density
muOPG met [22-194]	-
muOPG met [22-194] 5k PEG	+
muOPG met [22-194] 20k PEG	+
huOPG met [22-194] P25A	-
huOPG met [22-194] P25A 5k PEG	+
huOPG met [22-194] P25A 20k PEG	+
huOPG met [22-194] P25A 31k PEG	+
huOPG met [22-194] P25A 57k PEG	+
huOPG met [22-194] P25A 12k PEG	+
huOPG met [22-194] P25A 20k Branched PEG	+
huOPG met [22-194] P25A 8k PEG dimer	+
huOPG met [22-194] P25A disulfide crosslink	+

* * *

While the invention has been described in what is considered to be its preferred embodiments, it is not to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims, which scope is to be accorded the broadest interpretation so as to encompass all such modifications and equivalents.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:Amgen Inc.

(ii) TITLE OF INVENTION: OSTEOPROTEGERIN

(iii) NUMBER OF SEQUENCES: 168

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: United States
(F) ZIP: 91320

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Winter, Robert B.
(C) REFERENCE/DOCKET NUMBER: A-378-CIP2

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAAGGAAGGA AAAAAGCGGC CGCTACANNN NNNNNT 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCGACCCACG CGTCCG 16

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGTGCGCAG GC 12

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGTAAAACGA CGGCCAGT 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGGAAACAG CTATGACC 18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAATTAACCC TCACTAAAGG 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCATTATGAC CCAGAAACCG GAC 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGGTAGCGCC CTTCCTCACA TTC 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACTAGTCCC ACAATGAACA AGTGGCTGTG 30

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATAAGAATGC GGCCGCTAAA CTATGAAACA GCCCAGTGAC CATTC 45

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCTCTAGAA AGAGCTGGGA C 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCCGTGTTC CATTTATGAG C 21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATCAAAGGCA GGGCATACTT CCTG 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTTGCACTCC TGTTTCACGG TCTG 24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAAGACACCT TGAAGGGCCT GATG 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TAACTTTTAC AGAAGAGCAT CAGC 24

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGCGCGGCCG CATGAACAAG TGGCTGTGCT GCG 33

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGCTCTAGAG AAACAGCCCA GTGACCATTC C 31

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTGAAGCTGT GCAAGAACCT GATG 24

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATCAAAGGCA GGGCATACTT CCTG 24

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAGATCCTGA AGCTGCTCAG TTTG 24

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGCGCGGCCG CGGGGACCAC AATGAACAAG TTG 33

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGCTCTAGAA TTGTGAGGAA ACAGCTCAAT GGC 33

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATAGCGGCCG CTGAGCCCAA ATCTTGTGAC AAAACTCAC 39

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCTAGAGTCG ACTTATCATT TACCCGGAGA CAGGGAGAGG CTCTT 45

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG 38

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CCTCTGCGGC CGCTAAGCAG CTTATTTTCA CGGATTGAAC CTG 43

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG 38

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TCCGTAAGAA ACAGCCCAGT GACC 24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCTCTGCGGC CGCTGTTGCA TTTCCTTTCT G 31

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

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Glu Thr Leu Pro Pro Lys Tyr Leu His Tyr Asp Pro Glu Thr Gly His
1               5                   10                  15

Gln Leu Leu
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCCCTTGCCC TGACCACTCT T 21

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CCTCTGCGGC CGCACACACG TTGTCATGTG TTGC 34

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TCCCTTGCCC TGACCACTCT T 21

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCTCTGCGGC CGCCTTTTGC GTGGCTTCTC TGTT 34

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCTCTGAGCT CAAGCTTGGT TTCCGGGGAC CACAATG 37

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CCTCTGCGGC CGCTAAGCAG CTTATTTTTA CTGAATGG 38

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CCTCTGAGCT CAAGCTTGGT TTCCGGGGAC CACAATG 37

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CCTCTGCGGC CGCCAGGGTA ACATCTATTC CAC 33

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCGAAGCTTC CACCATGAAC AAGTGGCTGT GCTGC 35

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CCTCTGTCGA CTATTATAAG CAGCTTATTT TCACGGATTG 40

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TCCCTTGCCC TGACCACTCT T 21

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CCTCTGTCGA CTTAACACAC GTTGTCATGT GTTGC 35

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TCCCTTGCCC TGACCACTCT T 21

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CCTCTGTCGA CTTACTTTTG CGTGGCTTCT CTGTT 35

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1537 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GTGAAGAGCG TGAAGAGCGG TTCCTCCTTT CAGCAAAAAA CCCCTCAAGA CCCGTTTAGA 60

GGCCCCAAGG GGTTATGCTA GTTATTGCTC AGCGGTGGCA GCAGCCAACT CAGCTTCCTT 120

TCGGGCTTTC TTCTTCTTCT TCTTCTTTCC GCGGATCCTC GAGTAAGCTT CCATGGTACC 180

CTGCAGGTCG ACACTAGTGA GCTCGAATTC CAACGCGTTA ACCATATGTT ATTCCTCCTT 240

TAATTAGTTA AAACAAATCT AGAATCAAAT CGATTAATCG ACTATAACAA ACCATTTTCT 300

TGCGTAAACC TGTACGATCC TACAGGTACT TATGTTAAAC AATTGTATTT CAAGCGATAT 360

AATAGTGTGA CAAAAATCCA ATTTATTAGA ATCAAATGTC AATCTATTAC CGTTTTAATG 420

ATATATAACA CGCAAAACTT GCGACAAACA ATAGGTAAGG ATAAAGAGAT GGGTATGAAA 480

GACATAAATG CCGACGACAC TTACAGAATA ATTAATAAAA TTAAAGCCTG TAGAAGCAAT 540

AATGATATTA ATCAATGCTT ATCTGATATG ACTAAAATGG TACATTGTGA ATATTATTTA 600

CTCGCGATCA TTTATCCTCA TTCTATGGTT AAATCTGATA TTTCAATTCT GGATAATTAC 660

CCTAAAAAAT GGAGGCAATA TTATGATGAC GCTAATTTAA TAAAATATGA TCCTATAGTA 720

GATTATTCTA ACTCCAATCA TTCACCGATT AATTGGAATA TATTTGAAAA CAATGCTGTA 780

AATAAAAAAT CTCCAAATGT AATTAAAGAA GCGAAATCAT CAGGTCTTAT CACTGGGTTT 840

AGTTTCCCTA TTCATACTGC TAATAATGGC TTCGGAATGC TTAGTTTTGC ACATTCAGAG 900

AAAGACAACT ATATAGATAG TTTATTTTTA CATGCGTGTA TGAACATACC ATTAATTGTT 960

CCTTCTCTAG TTGATAATTA TCGAAAAATA AATATAGCAA ATAATAAATC AAACAACGAT 1020

TTAACCAAAA GAGAAAAAGA ATGTTTAGCG TGGGCATGCG AAGGAAAAAG CTCTTGGGAT 1080

ATTTCAAAAA TATTAGGCTG TAGTAAGCGC ACGGTCACTT TCCATTTAAC CAATGCGCAA 1140

ATGAAACTCA ATACAACAAA CCGCTGCCAA AGTATTTCTA AAGCAATTTT AACAGGAGCA 1200

ATTGATTGCC CATACTTTAA AAGTTAAGTA CGACGTCCAT ATTTGAATGT ATTTAGAAAA 1260

ATAAACAAAA GAGTTTGTAG AAACGCAAAA AGGCCATCCG TCAGGATGGC CTTCTGCTTA 1320

ATTTGATGCC TGGCAGTTTA TGGCGGGCGT CCTGCCCGCC ACCCTCCGGG CCGTTGCTTC 1380

GCAACGTTCA AATCCGCTCC CGGCGGATTT GTCCTACTCA GGAGAGCGTT CACCGACAAA 1440

CAACAGATAA AACGAAAGGC CCAGTCTTTC GACTGAGCCT TTCGTTTTAT TTGATGCCTG 1500

GCAGTTCCCT ACTCTCGCAT GGGGAGACCA TGCATAC 1537

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CCGGCGGACA TTTATCACAC AGCAGCTGAT GAGAAGTTTC TTCATCCA 48

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CGATTTGATT CTAGAAGGAG GAATAACATA TGGTTAACGC GTTGGAATTC GGTAC 55

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CGAATTCCAA CGCGTTAACC ATATGTTATT CCTCCTTCTA GAATCAAAT 49

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1546 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

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GCGTAACGTA TGCATGGTCT CCCCATGCGA GAGTAGGGAA CTGCCAGGCA TCAAATAAAA     60
CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT CGTTTTATCT GTTGTTTGTC GGTGAACGCT    120
CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG TTGCGAAGCA ACGGCCCGGA    180
GGGTGGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC AAATTAAGCA GAAGGCCATC    240
CTGACGGATG GCCTTTTTGC GTTTCTACAA ACTCTTTTGT TTATTTTTCT AAATACATTC    300
AAATATGGAC GTCGTACTTA ACTTTTAAAG TATGGGCAAT CAATTGCTCC TGTTAAAATT    360
GCTTTAGAAA TACTTTGGCA GCGGTTTGTT GTATTGAGTT TCATTTGCGC ATTGGTTAAA    420
TGGAAAGTGA CCGTGCGCTT ACTACAGCCT AATATTTTTG AAATATCCCA AGAGCTTTTT    480
CCTTCGCATG CCCACGCTAA ACATTCTTTT TCTCTTTTGG TTAAATCGTT GTTTGATTTA    540
TTATTTGCTA TATTTATTTT TCGATAATTA TCAACTAGAG AAGGAACAAT TAATGGTATG    600
TTCATACACG CATGTAAAAA TAAACTATCT ATATAGTTGT CTTTCTCTGA ATGTGCAAAA    660
CTAAGCATTC CGAAGCCATT ATTAGCAGTA TGAATAGGGA AACTAAACCC AGTGATAAGA    720
CCTGATGATT TCGCTTCTTT AATTACATTT GGAGATTTTT TATTTACAGC ATTGTTTTCA    780
AATATATTCC AATTAATCGG TGAATGATTG GAGTTAGAAT AATCTACTAT AGGATCATAT    840
TTTATTAAAT TAGCGTCATC ATAATATTGC CTCCATTTTT TAGGGTAATT ATCCAGAATT    900
GAAATATCAG ATTTAACCAT AGAATGAGGA TAAATGATCG CGAGTAAATA ATATTCACAA    960
TGTACCATTT TAGTCATATC AGATAAGCAT TGATTAATAT CATTATTGCT TCTACAGGCT   1020
TTAATTTTAT TAATTATTCT GTAAGTGTCG TCGGCATTTA TGTCTTTCAT ACCCATCTCT   1080
TTATCCTTAC CTATTGTTTG TCGCAAGTTT TGCGTGTTAT ATATCATTAA AACGGTAATA   1140
GATTGACATT TGATTCTAAT AAATTGGATT TTTGTCACAC TATTATATCG CTTGAAATAC   1200
AATTGTTTAA CATAAGTACC TGTAGGATCG TACAGGTTTA CGCAAGAAAA TGGTTTGTTA   1260
TAGTCGATTA ATCGATTTGA TTCTAGATTT GTTTTAACTA ATTAAAGGAG GAATAACATA   1320
TGGTTAACGC GTTGGAATTC GAGCTCACTA GTGTCGACCT GCAGGGTACC ATGGAAGCTT   1380
ACTCGAGGAT CCGCGGAAAG AAGAAGAAGA AGAAGAAAGC CCGAAAGGAA GCTGAGTTGG   1440
CTGCTGCCAC CGCTGAGCAA TAACTAGCAT AACCCCTTGG GGCCTCTAAA CGGGTCTTGA   1500
```

GGGGTTTTTT GCTGAAAGGA GGAACCGCTC TTCACGCTCT TCACGC 1546

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TATGAAACAT CATCACCATC ACCATCATGC TAGCGTTAAC GCGTTGG 47

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AATTCCAACG CGTTAACGCT AGCATGATGG TGATGGTGAT GATGTTTCA 49

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CTAATTCCGC TCTCACCTAC CAAACAATGC CCCCCTGCAA AAAATAAATT CATATAAAAA 60

ACATACAGAT AACCATCTGC GGTGATAAAT TATCTCTGGC GGTGTTGACA TAAATACCAC 120

TGGCGGTGAT ACTGAGCACA T 141

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CGATGTGCTC AGTATCACCG CCAGTGGTAT TTATGTCAAC ACCGCCAGAG ATAATTTATC 60

ACCGCAGATG GTTATCTGTA TGTTTTTTAT ATGAATTTAT TTTTTGCAGG GGGGCATTGT 120

TTGGTAGGTG AGAGCGGAAT TAGACGT 147

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CGATTTGATT CTAGAAGGAG GAATAACATA TGGTTAACGC GTTGGAATTC GGTAC 55

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CGAATTCCAA CGCGTTAACC ATATGTTATT CCTCCTTCTA GAATCAAAT 49

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 668 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GTGAAGAGCG TGAAGAGCGG TTCCTCCTTT CAGCAAAAAA CCCCTCAAGA CCCGTTTAGA 60

GGCCCCAAGG GGTTATGCTA GTTATTGCTC AGCGGTGGCA GCAGCCAACT CAGCTTCCTT 120

TCGGGCTTTC TTCTTCTTCT TCTTCTTTCC GCGGATCCTC GAGTAAGCTT CCATGGTACC 180

CTGCAGGTCG ACACTAGTGA GCTCGAATTC CAACGCGTTA ACCATATGTT ATTCCTCCTT 240

TAATTAGTTA ACTCAAATCT AGAATCAAAT CGATAAATTG TGAGCGCTCA CAATTGAGAA 300

TATTAATCAA GAATTTTAGC ATTTGTCAAA TGAATTTTTT AAAAATTATG AGACGTCCAT 360

ATTTGAATGT ATTTAGAAAA ATAAACAAAA GAGTTTGTAG AAACGCAAAA AGGCCATCCG 420

TCAGGATGGC CTTCTGCTTA ATTTGATGCC TGGCAGTTTA TGGCGGGCGT CCTGCCCGCC 480

ACCCTCCGGG CCGTTGCTTC GCAACGTTCA AATCCGCTCC CGGCGGATTT GTCCTACTCA 540

GGAGAGCGTT CACCGACAAA CAACAGATAA AACGAAAGGC CCAGTC[illegible]TC GACTGAGCCT 600

TTCGTTTTAT TTGATGCCTG GCAGTTCCCT ACTCTCGCAT GGGGAGACCA TGCATACGTT 660

ACGCACGT 668

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 726 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GCGTAACGTA TGCATGGTCT CCCCATGCGA GAGTAGGGAA CTGCCAGGCA TCAAATAAAA 60

CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT CGTTTTATCT GTTGTTTGTC GGTGAACGCT 120

CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG TTGCGAAGCA ACGGCCCGGA 180

GGGTGGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC AAATTAAGCA GAAGGGGCCT 240

CCCACCGCCC GTCCTGCGGG CGGTATTTGA CGGTCCGTAG TTTAATTCGT CTTCGCCATC 300

CTGACGGATG GCCTTTTTGC GTTTCTACAA ACTCTTTTGT TTATTTTTCT AAATACATTC 360

AAATATGGAC GTCTCATAAT TTTTAAAAAA TTCATTTGAC AAATGCTAAA ATTCTTGATT 420

AATATTCTCA ATTGTGAGCG CTCACAATTT ATCGATTTGA TTCTAGATTT GTTTTAACTA 480

ATTAAAGGAG GAATAACATA TGGTTAACGC GTTGGAATTC GAGCTCACTA GTGTCGACCT 540

GCAGGGTACC ATGGAAGCTT ACTCGAGGAT CCGCGGAAAG AAGAAGAAGA AGAAGAAAGC 600

CCGAAAGGAA GCTGAGTTGG CTGCTGCCAC CGCTGAGCAA TAACTAGCAT AACCCCTTGG 660

GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GCTGAAAGGA GGAACCGCTC TTCACGCTCT 720

TCACGC 726

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TACGCACTGG ATCCTTATAA GCAGCTTATT TTTACTGATT GGAC 44

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GTCCTCCTGG TACCTACCTA AAACAAC 27

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TATGGATGAA GAAACTTCTC ATCAGCTGCT GTGTGATAAA TGTCCGCCGG GTACCCGGCG 60

GACATTTATC ACACAGCAGC TGATGAGAAG TTTCTTCATC CA 102

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Met Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro
1 5 10 15

Gly Thr Tyr

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TATGGAAACT TTTCCTCCAA AATATCTTCA TTATGATGAA GAAACTTCTC ATCAGCTGCT 60

GTGTGATAAA TGTCCGCCGG GTAC 84

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CCGGCGGACA TTTATCACAC AGCAGCTGAT GAGAAGTTTC TTCATCATAA TGAAGATATT 60

TTGGAGGAAA AGTTTCCA 78

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

TACGCACTGG ATCCTTATAA GCAGCTTATT TTCACGGATT GAAC 44

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GTGCTCCTGG TACCTACCTA AAACAGCACT GCACAGTG 38

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TATGGAAACT CTGCCTCCAA AATACCTGCA TTACGATCCG GAAACTGGTC ATCAGCTGCT 60

GTGTGATAAA TGTGCTCCGG GTAC 84

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC CGGATCGTAA TGCAGGTATT 60

TTGGAGGCAG AGTTTCCA 78

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

TATGGACCCA GAAACTGGTC ATCAGCTGCT GTGTGATAAA TGTGCTCCGG GTAC 54

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC TGGGTCCA 48

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

TATGAAAGAA ACTCTGCCTC CAAAATACCT GCATTACGAT CCGGAAACTG GTCATCAGCT 60

GCTGTGTGAT AAATGTGCTC CGGGTAC 87

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC CGGATCGTAA TGCAGGTATT 60

TTGGAGGCAG AGTTTCTTTC A 81

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GTTCTCCTCA TATGAAACAT CATCACCATC ACCATCATGA AACTCTGCCT CCAAAATACC 60

TGCATTACGA T 71

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GTTCTCCTCA TATGAAAGAA ACTCTGCCTC CAAAATACCT GCA 43

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

TACGCACTGG ATCCTTAATG ATGGTGATGG TGATGATGTA AGCAGCTTAT TTTCACGGAT 60

TGAACCTGAT TCCCTA 76

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GTTCTCCTCA TATGAAATAC CTGCATTACG ATCCGGAAAC TGGTCAT 47

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GTTCTCCTAT TAATGAAATA TCTTCATTAT GATGAAGAAA CTT 43

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

TACGCACTGG ATCCTTATAA GCAGCTTATT TTTACTGATT 40

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GTTCTCCTCA TATGGAAACT CTGCCTCCAA AATACCTGCA 40

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TACGCACTGG ATCCTTATGT TGCATTTCCT TTCTGAATTA GCA 43

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

CCGGAAACAG ATAATGAG 18

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GATCCTCATT ATCTGTTT 18

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CCGGAAACAG AGAAGCCACG CAAAAGTAAG 30

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GATCCTTACT TTTGCGTGGC TTCTCTGTTT 30

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TATGTTAATG AG 12

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GATCCTCATT AACA 14

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TATGTTCCGG AAACAGTTAA G 21

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GATCCTTAAC TGTTTCCGGA ACA 23

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

TATGTTCCGG AAACAGTGAA TCAACTCAAA AATAAG 36

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GATCCTTATT TTTGAGTTGA TTCACTGTTT CCGGAACA 38

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CTAGCGACGA CGACGACAAA GAAACTCTGC CTCCAAAATA CCTGCATTAC GATCCGGAAA 60

CTGGTCATCA GCTGCTGTGT GATAAATGTG CTCCGGGTAC 100

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC CGGATCGTAA TGCAGGTATT 60

TTGGAGGCAG AGTTTCTTTG TCGTCGTCGT CG 92

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

ACAAACACAA TCGATTTGAT ACTAGA 26

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TTTGTTTTAA CTAATTAAAG GAGGAATAAA ATATGAGAGG ATCGCATCAC 50

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

CATCACCATC ACGAAACCTT CCCGCCGAAA TACCTGCACT ACGACGAAGA 50

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

AACCTCCCAC CAGCTGCTGT GCGACAAATG CCCGCCGGGT ACCCAAACA 49

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

TGTTTGGGTA CCCGGCGGGC ATTTGT 26

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CGCACAGCAG CTGGTGGGAG GTTTCTTCGT CGTAGTGCAG GTATTTCGGC 50

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

GGGAAGGTTT CGTGATGGTG ATGGTGATGC GATCCTCTCA TATTTTATT 49

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CCTCCTTTAA TTAGTTAAAA CAAATCTAGT ATCAAATCGA TTGTGTTTGT 50

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

ACAAACACAA TCGATTTGAT ACTAGATTTG TTTTAACTAA TTAAAGGAGG AATAAAATG 59

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

CTAATTAAAG GAGGAATAAA ATGAAAGAAA CTTTTCCTCC AAAATATC 48

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

TGTTTGGGTA CCCGGCGGAC ATTTATCACA C 31

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

ACAAACACAA TCGATTTGAT ACTAGATTTG TTTTAACTAA TTAAAGGAGG AATAAAATG 59

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

CTAATTAAAG GAGGAATAAA ATGAAAAAAA AAGAAACTTT TCCTCCAAAA TATC 54

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

TGTTTGGGTA CCCGGCGGAC ATTTATCACA C 31

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

CAGCCCGGGT AAAATGGAAA CGTTTCCTCC AAAATATCTT CATT 44

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

CGTTTCCATT TTACCCGGGC TGAGCGAGAG GCTCTTCTGC GTGT 44

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

CGCTCAGCCC GGGTAAAATG GAAACGTTGC CTCCAAAATA CCTGC 45

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

CCATTTTACC CGGGCTGAGC GAGAGGCTCT TCTGCGTGT 39

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

GAAAATAAGC TGCTTAGCTG CAGCTGAACC AAAATC 36

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

CAGCTGCAGC TAAGCAGCTT ATTTTCACGG ATTG 34

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

AAAAATAAGC TGCTTAGCTG CAGCTGAACC AAAATC 36

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

CAGCTGCAGC TAAGCAGCTT ATTTTTACTG ATTGG 35

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

CTAGAAGGAG GAATAACATA TGGAAACTTT TGCTCCAAAA TATCTTCATT ATGATGAAGA 60

AACTAGTCAT CAGCTGCTGT GTGATAAATG TCCGCCGGGT AC 102

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

CCGGCGGACA TTTATCACAC AGCAGCTGAT GACTAGTTTC TTCATCATAA TGAAGATATT 60

TTGGAGCAAA AGTTTCCATA TGTTATTCCT CCTT 94

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

CTAGAAGGAG GAATAACATA TGGAAACTTT TCCTGCTAAA TATCTTCATT ATGATGAAGA 60

AA 62

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

CTAGTTTCTT CATCATAATG AAGATATTTA GCAGGAAAAG TTTCCATATG TTATTCCTCC 60

TT 62

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

```
Tyr His Tyr Tyr Asp Gln Asn Gly Arg Met Cys Glu Glu Cys His Met
1               5                   10                  15

Cys Gln Pro Gly His Phe Leu Val Lys His Cys Lys Gln Pro Lys Arg
            20                  25                  30

Asp Thr Val Cys His Lys Pro Cys Glu Pro Gly Val Thr Tyr Thr Asp
        35                  40                  45

Asp Trp His
    50
```

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 124..1326

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

```
ATCAAAGGCA GGGCATACTT CCTGTTGCCC AGACCTTATA TAAAACGTCA TGTTCGCCTG      60

GGCAGCAGAG AAGCACCTAG CACTGGCCCA GCGGCTGCCG CCTGAGGTTT CCAGAGGACC     120

ACA ATG AAC AAG TGG CTG TGC TGT GCA CTC CTG GTG TTC TTG GAC ATC       168
    Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Phe Leu Asp Ile
      1               5                  10                  15

ATT GAA TGG ACA ACC CAG GAA ACC TTT CCT CCA AAA TAC TTG CAT TAT       216
Ile Glu Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr
                20                  25                  30
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GAC CCA GAA ACC GGA CGT CAG CTC TTG TGT GAC AAA TGT GCT CCT GGC     264
Asp Pro Glu Thr Gly Arg Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly
         35                  40                  45

ACC TAC CTA AAA CAG CAC TGC ACA GTC AGG AGG AAG ACA CTG TGT GTC     312
Thr Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val
     50                  55                  60

CCT TGC CCT GAC TAC TCT TAT ACA GAC AGC TGG CAC ACG AGT GAT GAA     360
Pro Cys Pro Asp Tyr Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu
    65                  70                  75

TGC GTG TAC TGC AGC CCC GTG TGC AAG GAA CTG CAG ACC GTG AAA CAG     408
Cys Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Thr Val Lys Gln
80                  85                  90                  95

GAG TGC AAC CGC ACC CAC AAC CGA GTG TGC GAA TGT GAG GAA GGG CGC     456
Glu Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg
                100                 105                 110

TAC CTG GAG CTC GAA TTC TGC TTG AAG CAC CGG AGC TGT CCC CCA GGC     504
Tyr Leu Glu Leu Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly
            115                 120                 125

TTG GGT GTG CTG CAG GCT GGG ACC CCA GAG CGA AAC ACG GTT TGC AAA     552
Leu Gly Val Leu Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys
        130                 135                 140

AGA TGT CCG GAT GGG TTC TTC TCA GGT GAG ACG TCA TCG AAA GCA CCC     600
Arg Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro
    145                 150                 155

TGT AGG AAA CAC ACC AAC TGC AGC TCA CTT GGC CTC CTG CTA ATT CAG     648
Cys Arg Lys His Thr Asn Cys Ser Ser Leu Gly Leu Leu Leu Ile Gln
160                 165                 170                 175

AAA GGA AAT GCA ACA CAT GAC AAT GTA TGT TCC GGA AAC AGA GAA GCA     696
Lys Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala
                180                 185                 190

ACT CAA AAT TGT GGA ATA GAT GTC ACC CTG TGC GAA GAG GCA TTC TTC     744
Thr Gln Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe
            195                 200                 205

AGG TTT GCT GTG CCT ACC AAG ATT ATA CCG AAT TGG CTG AGT GTT CTG     792
Arg Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu
        210                 215                 220

GTG GAC AGT TTG CCT GGG ACC AAA GTG AAT GCA GAG AGT GTA GAG AGG     840
Val Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg
    225                 230                 235
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ATA AAA CGG AGA CAC AGC TCG CAA GAG CAA ACT TTC CAG CTA CTT AAG        888
Ile Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys
240                 245                 250                 255

CTG TGG AAG CAT CAA AAC AGA GAC CAG GAA ATG GTG AAG AAG ATC ATC        936
Leu Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile
                260                 265                 270

CAA GAC ATT GAC CTC TGT GAA AGC AGT GTG CAA CGG CAT ATC GGC CAC        984
Gln Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Ile Gly His
            275                 280                 285

GCG AAC CTC ACC ACA GAG CAG CTC CGC ATC TTG ATG GAG AGC TTG CCT       1032
Ala Asn Leu Thr Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pro
        290                 295                 300

GGG AAG AAG ATC AGC CCA GAC GAG ATT GAG AGA ACG AGA AAG ACC TGC       1080
Gly Lys Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys
    305                 310                 315

AAA CCC AGC GAG CAG CTC CTG AAG CTA CTG AGC TTG TGG AGG ATC AAA       1128
Lys Pro Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys
320                 325                 330                 335

AAT GGA GAC CAA GAC ACC TTG AAG GGC CTG ATG TAC GCA CTC AAG CAC       1176
Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His
                340                 345                 350

TTG AAA GCA TAC CAC TTT CCC AAA ACC GTC ACC CAC AGT CTG AGG AAG       1224
Leu Lys Ala Tyr His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys
            355                 360                 365

ACC ATC AGG TTC TTG CAC AGC TTC ACC ATG TAC CGA TTG TAT CAG AAA       1272
Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys
        370                 375                 380

CTC TTT CTA GAA ATG ATA GGG AAT CAG GTT CAA TCA GTG AAG ATA AGC       1320
Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser
    385                 390                 395

TGC TTA TAGTTAGGAA TGGTCACTGG GCTGTTTCTT CAGGATGGGC CAACACTGAT        1376
Cys Leu
400

GGAGCAGATG GCTGCTTCTC CGGCTCTTGA AATGGCAGTT GATTCCTTTC TCATCAGTTG     1436

GTGGGAATGA AGATCCTCCA GCCCAACACA CACACTGGGG AGTCTGAGTC AGGAGAGTGA     1496

GGCAGGCTAT TTGATAATTG TGCAAAGCTG CCAGGTGTAC ACCTAGAAAG TCAAGCACCC     1556
```

TGAGAAAGAG GATATTTTTA TAACCTCAAA CATAGGCCCT TTCCTTCCTC TCCTTATGGA 1616

TGAGTACTCA GAAGGCTTCT ACTATCTTCT GTGTCATCCC TAGATGAAGG CCTCTTTTAT 1676

TTATTTTTTT ATTCTTTTTT TCGGAGCTGG GGACCGAACC CAGGGCCTTG CGCTTGCGAG 1736

GCAAGTGCTC TACCACTGAG CTAAATCTCC AACCCCTGAA GGCCTCTTTC TTTCTGCCTC 1796

TGATAGTCTA TGACATTCTT TTTTCTACAA TTCGTATCAG GTGCACGAGC CTTATCCCAT 1856

TTGTAGGTTT CTAGGCAAGT TGACCGTTAG CTATTTTTCC CTCTGAAGAT TTGATTCGAG 1916

TTGCAGACTT GGCTAGACAA GCAGGGGTAG GTTATGGTAG TTTATTTAAC AGACTGCCAC 1976

CAGGAGTCCA GTGTTTCTTG TTCCTCTGTA GTTGTACCTA AGCTGACTCC AAGTACATTT 2036

AGTATGAAAA ATAATCAACA AATTTTATTC CTTCTATCAA CATTGGCTAG CTTTGTTTCA 2096

GGGCACTAAA AGAAACTACT ATATGGAGAA AGAATTGATA TTGCCCCCAA CGTTCAACAA 2156

CCCAATAGTT TATCCAGCTG TCATGCCTGG TTCAGTGTCT ACTGACTATG CGCCCTCTTA 2216

TTACTGCATG CAGTAATTCA ACTGGAAATA GTAATAATAA TAATAGAAAT AAAATCTAGA 2276

CTCCATTGGA TCTCTCTGAA TATGGGAATA TCTAACTTAA GAAGCTTTGA GATTTCAGTT 2336

GTGTTAAAGG CTTTTATTAA AAAGCTGATG CTCTTCTGTA AAAGTTACTA ATATATCTGT 2396

AAGACTATTA CAGTATTGCT ATTTATATCC ATCCAG 2432

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 401 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Phe Leu Asp Ile Ile
1 5 10 15

Glu Trp Thr Thr Gln Glu Thr Phe Pro Pr Lys Tyr Leu His Tyr Asp
20 25 30

Pro Glu Thr Gly Arg Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
35 40 45

Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
50 55 60

Cys Pro Asp Tyr Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65 70 75 80

Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Thr Val Lys Gln Glu
85 90 95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr
100 105 110

Leu Glu Leu Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Leu
115 120 125

Gly Val Leu Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg
130 135 140

Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145 150 155 160

Arg Lys His Thr Asn Cys Ser Ser Leu Gly Leu Leu Leu Ile Gln Lys
165 170 175

Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
180 185 190

Gln Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
195 200 205

Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val
210 215 220

Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225 230 235 240

Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
245 250 255

Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln
260 265 270

Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Ile Gly His Ala
275 280 285

Asn Leu Thr Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pr Gly
290 295 300

```
Lys Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys
305                 310                 315                 320

Pro Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
                325                 330                 335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu
            340                 345                 350

Lys Ala Tyr His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr
        355                 360                 365

Ile Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu
    370                 375                 380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385                 390                 395                 400

Leu
```

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1324 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 90..1292

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

```
CCTTATATAA ACGTCATGAT TGCCTGGGCT GCAGAGACGC ACCTAGCACT GACCCAGCGG        60

CTGCCTCCTG AGGTTTCCCG AGGACCACA ATG AAC AAG TGG CTG TGC TGC GCA        113
                                Met Asn Lys Trp Leu Cys Cys Ala
                                  1               5

CTC CTG GTG CTC CTG GAC ATC ATT GAA TGG ACA ACC CAG GAA ACC CTT        161
Leu Leu Val Leu Leu Asp Ile Ile Glu Trp Thr Thr Gln Glu Thr Leu
     10                  15                  20
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```
CCT CCA AAG TAC TTG CAT TAT GAC CCA GAA ACT GGT CAT CAG CTC CTG      209
Pro Pro Lys Tyr Leu His Tyr Asp Pro Glu Thr Gly His Gln Leu Leu
 25                  30                  35                  40

TGT GAC AAA TGT GCT CCT GGC ACC TAC CTA AAA CAG CAC TGC ACA GTG      257
Cys Asp Lys Cys Ala Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Val
                 45                  50                  55

AGG AGG AAG ACA TTG TGT GTC CCT TGC CCT GAC CAC TCT TAT ACG GAC      305
Arg Arg Lys Thr Leu Cys Val Pro Cys Pro Asp His Ser Tyr Thr Asp
             60                  65                  70

AGC TGG CAC ACC AGT GAT GAG TGT GTG TAT TGC AGC CCA GTG TGC AAG      353
Ser Trp His Thr Ser Asp Glu Cys Val Tyr Cys Ser Pro Val Cys Lys
         75                  80                  85

GAA CTG CAG TCC GTG AAG CAG GAG TGC AAC CGC ACC CAC AAC CGA GTG      401
Glu Leu Gln Ser Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val
     90                  95                 100

TGT GAG TGT GAG GAA GGG CGT TAC CTG GAG ATC GAA TTC TGC TTG AAG      449
Cys Glu Cys Glu Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
105                 110                 115                 120

CAC CGG AGC TGT CCC CCG GGC TCC GGC GTG GTG CAA GCT GGA ACC CCA      497
His Arg Ser Cys Pro Pro Gly Ser Gly Val Val Gln Ala Gly Thr Pro
                125                 130                 135

GAG CGA AAC ACA GTT TGC AAA AAA TGT CCA GAT GGG TTC TTC TCA GGT      545
Glu Arg Asn Thr Val Cys Lys Lys Cys Pro Asp Gly Phe Phe Ser Gly
            140                 145                 150

GAG ACT TCA TCG AAA GCA CCC TGT ATA AAA CAC ACG AAC TGC AGC ACA      593
Glu Thr Ser Ser Lys Ala Pro Cys Ile Lys His Thr Asn Cys Ser Thr
        155                 160                 165

TTT GGC CTC CTG CTA ATT CAG AAA GGA AAT GCA ACA CAT GAC AAC GTG      641
Phe Gly Leu Leu Leu Ile Gln Lys Gly Asn Ala Thr His Asp Asn Val
    170                 175                 180

TGT TCC GGA AAC AGA GAA GCC ACG CAA AAG TGT GGA ATA GAT GTC ACC      689
Cys Ser Gly Asn Arg Glu Ala Thr Gln Lys Cys Gly Ile Asp Val Thr
185                 190                 195                 200

CTG TGT GAA GAG GCC TTC TTC AGG TTT GCT GTT CCT ACC AAG ATT ATA      737
Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Ile Ile
                205                 210                 215
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CCA AAT TGG CTG AGT GTT TTG GTG GAC AGT TTG CCT GGG ACC AAA GTG       785
Pro Asn Trp Leu Ser Val Leu Val Asp Ser Leu Pro Gly Thr Lys Val
            220                 225                 230

AAT GCC GAG AGT GTA GAG AGG ATA AAA CGG AGA CAC AGC TCA CAA GAG       833
Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Arg His Ser Ser Gln Glu
        235                 240                 245

CAA ACC TTC CAG CTG CTG AAG CTG TGG AAA CAT CAA AAC AGA GAC CAG       881
Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Arg Asp Gln
    250                 255                 260

GAA ATG GTG AAG AAG ATC ATC CAA GAC ATT GAC CTC TGT GAA AGC AGC       929
Glu Met Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Ser Ser
265                 270                 275                 280

GTG CAG CGG CAT CTC GGC CAC TCG AAC CTC ACC ACA GAG CAG CTT CTT       977
Val Gln Arg His Leu Gly His Ser Asn Leu Thr Thr Glu Gln Leu Leu
                285                 290                 295

GCC TTG ATG GAG AGC CTG CCT GGG AAG AAG ATC AGC CCA GAA GAG ATT      1025
Ala Leu Met Glu Ser Leu Pro Gly Lys Lys Ile Ser Pro Glu Glu Ile
            300                 305                 310

GAG AGA ACG AGA AAG ACC TGC AAA TCG AGC GAG CAG CTC CTG AAG CTA      1073
Glu Arg Thr Arg Lys Thr Cys Lys Ser Ser Glu Gln Leu Leu Lys Leu
        315                 320                 325

CTC AGT TTA TGG AGG ATC AAA AAT GGT GAC CAA GAC ACC TTG AAG GGC      1121
Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly
    330                 335                 340

CTG ATG TAT GCC CTC AAG CAC TTG AAA ACA TCC CAC TTT CCC AAA ACT      1169
Leu Met Tyr Ala Leu Lys His Leu Lys Thr Ser His Phe Pro Lys Thr
345                 350                 355                 360

GTC ACC CAC AGT CTG AGG AAG ACC ATG AGG TTC CTG CAC AGC TTC ACA      1217
Val Thr His Ser Leu Arg Lys Thr Met Arg Phe Leu His Ser Phe Thr
                365                 370                 375

ATG TAC AGA CTG TAT CAG AAG CTC TTT TTA GAA ATG ATA GGG AAT CAG      1265
Met Tyr Arg Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln
            380                 385                 390

GTT CAA TCC GTG AAA ATA AGC TGC TTA TAACTAGGAA TGGTCACTGG            1312
Val Gln Ser Val Lys Ile Ser Cys Leu
        395                 400

GCTGTTTCTT CA                                                        1324
```

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 401 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

```
Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu Asp Ile Ile
 1               5                  10                  15

Glu Trp Thr Thr Gln Glu Thr Leu Pro Pro Lys Tyr Leu His Tyr Asp
            20                  25                  30

Pro Glu Thr Gly His Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
        35                  40                  45

Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
    50                  55                  60

Cys Pro Asp His Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65                  70                  75                  80

Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Ser Val Lys Gln Glu
                85                  90                  95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr
            100                 105                 110

Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Ser
        115                 120                 125

Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Lys
    130                 135                 140

Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145                 150                 155                 160

Ile Lys His Thr Asn Cys Ser Thr Phe Gly Leu Leu Leu Ile Gln Lys
                165                 170                 175

Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
            180                 185                 190

Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
        195                 200                 205
```

Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val
210 215 220

Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225 230 235 240

Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
245 250 255

Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln
260 265 270

Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser
275 280 285

Asn Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly
290 295 300

Lys Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys
305 310 315 320

Ser Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
325 330 335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu
340 345 350

Lys Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr
355 360 365

Met Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu
370 375 380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385 390 395 400

Leu

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1355 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 94..1296

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

```
GTATATATAA CGTGATGAGC GTACGGGTGC GGAGACGCAC CGGAGCGCTC GCCCAGCCGC        60

CGCTCCAAGC CCCTGAGGTT TCCGGGGACC ACA ATG AAC AAG TTG CTG TGC TGC      114
                                     Met Asn Lys Leu Leu Cys Cys
                                       1               5

GCG CTC GTG TTT CTG GAC ATC TCC ATT AAG TGG ACC ACC CAG GAA ACG      162
Ala Leu Val Phe Leu Asp Ile Ser Ile Lys Trp Thr Thr Gln Glu Thr
         10                  15                  20

TTT CCT CCA AAG TAC CTT CAT TAT GAC GAA GAA ACC TCT CAT CAG CTG      210
Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His Gln Leu
     25                  30                  35

TTG TGT GAC AAA TGT CCT CCT GGT ACC TAC CTA AAA CAA CAC TGT ACA      258
Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His Cys Thr
 40                  45                  50                  55

GCA AAG TGG AAG ACC GTG TGC GCC CCT TGC CCT GAC CAC TAC TAC ACA      306
Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr
                 60                  65                  70

GAC AGC TGG CAC ACC AGT GAC GAG TGT CTA TAC TGC AGC CCC GTG TGC      354
Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys
             75                  80                  85

AAG GAG CTG CAG TAC GTC AAG CAG GAG TGC AAT CGC ACC CAC AAC CGC      402
Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg
         90                  95                 100

GTG TGC GAA TGC AAG GAA GGG CGC TAC CTT GAG ATA GAG TTC TGC TTG      450
Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu
    105                 110                 115

AAA CAT AGG AGC TGC CCT CCT GGA TTT GGA GTG GTG CAA GCT GGA ACC      498
Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
120                 125                 130                 135

CCA GAG CGA AAT ACA GTT TGC AAA AGA TGT CCA GAT GGG TTC TTC TCA      546
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe Ser
                140                 145                 150
```

```
AAT GAG ACG TCA TCT AAA GCA CCC TGT AGA AAA CAC ACA AAT TGC AGT        594
Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn Cys Ser
            155                 160                 165

GTC TTT GGT CTC CTG CTA ACT CAG AAA GGA AAT GCA ACA CAC GAC AAC        642
Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn
        170                 175                 180

ATA TGT TCC GGA AAC AGT GAA TCA ACT CAA AAA TGT GGA ATA GAT GTT        690
Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val
    185                 190                 195

ACC CTG TGT GAG GAG GCA TTC TTC AGG TTT GCT GTT CCT ACA AAG TTT        738
Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe
200                 205                 210                 215

ACG CCT AAC TGG CTT AGT GTC TTG GTA GAC AAT TTG CCT GGC ACC AAA        786
Thr Pro Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys
                220                 225                 230

GTA AAC GCA GAG AGT GTA GAG AGG ATA AAA CGG CAA CAC AGC TCA CAA        834
Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln
            235                 240                 245

GAA CAG ACT TTC CAG CTG CTG AAG TTA TGG AAA CAT CAA AAC AAA GCC        882
Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Ala
        250                 255                 260

CAA GAT ATA GTC AAG AAG ATC ATC CAA GAT ATT GAC CTC TGT GAA AAC        930
Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn
    265                 270                 275

AGC GTG CAG CGG CAC ATT GGA CAT GCT AAC CTC ACC TTC GAG CAG CTT        978
Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu
280                 285                 290                 295

CGT AGC TTG ATG GAA AGC TTA CCG GGA AAG AAA GTG GGA GCA GAA GAC       1026
Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp
                300                 305                 310

ATT GAA AAA ACA ATA AAG GCA TGC AAA CCC AGT GAC CAG ATC CTG AAG       1074
Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys
            315                 320                 325

CTG CTC AGT TTG TGG CGA ATA AAA AAT GGC GAC CAA GAC ACC TTG AAG       1122
Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys
        330                 335                 340
```

```
GGC CTA ATG CAC GCA CTA AAG CAC TCA AAG ACG TAC CAC TTT CCC AAA      1170
Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys
    345                 350                 355

ACT GTC ACT CAG AGT CTA AAG AAG ACC ATC AGG TTC CTT CAC AGC TTC      1218
Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe
360                 365                 370                 375

ACA ATG TAC AAA TTG TAT CAG AAG TTA TTT TTA GAA ATG ATA GGT AAC      1266
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly Asn
                380                 385                 390

CAG GTC CAA TCA GTA AAA ATA AGC TGC TTA TAACTGGAAA TGGCCATTGA        1316
Gln Val Gln Ser Val Lys Ile Ser Cys Leu
            395                 400

GCTGTTTCCT CACAATTGGC GAGATCCCAT GGATGATAA                           1355
```

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 401 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

```
Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser Ile
  1               5                  10                  15

Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp
            20                  25                  30

Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr
        35                  40                  45

Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro
    50                  55                  60

Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
 65                  70                  75                  80

Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu
                85                  90                  95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr
            100                 105                 110
```

Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe
115 120 125

Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg
130 135 140

Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys
145 150 155 160

Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys
165 170 175

Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr
180 185 190

Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
195 200 205

Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val
210 215 220

Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225 230 235 240

Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
245 250 255

Trp Lys His Gln Asn Lys Ala Gln Asp Ile Val Lys Lys Ile Ile Gln
260 265 270

Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala
275 280 285

Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly
290 295 300

Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys
305 310 315 320

Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
325 330 335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser
340 345 350

Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr
355 360 365

Ile Arg Phe Leu His Ser Phe Thr M t Tyr Lys Leu Tyr Gln Lys Leu
370 375 380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385 390 395 400

Leu

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys
1 5 10 15

Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro
20 25 30

Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala
35 40 45

Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys
50 55 60

Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr
65 70 75 80

Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn
85 90 95

Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His
100 105 110

Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly
115 120 125

Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys
130 135

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

CCGGCGGACA TTTATCACAC AGCAGCTGAT GAGAAGTTTC TTCATCCA 48

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

```
Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala
1               5                   10                  15

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
            20                  25                  30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn
        35                  40                  45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
    50                  55                  60

Pr  Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65                  70                  75                  80
```

```
Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
                85                  90                  95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
            100                 105                 110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
        115                 120                 125

Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
    130                 135                 140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145                 150                 155                 160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
                165                 170                 175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
            180                 185                 190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
        195                 200                 205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr
    210                 215
```

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 280 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

```
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1               5                   10                  15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
            20                  25                  30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
        35                  40                  45
```

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50   55   60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65   70   75   80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
                85   90   95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
            100   105   110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
        115   120   125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130   135   140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145   150   155   160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165   170   175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
            180   185   190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
        195   200   205

Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
    210   215   220

Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
225   230   235   240

Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
                245   250   255

Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
            260   265   270

Phe Ser Pro Thr Pro Gly Phe Thr
        275   280

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

```
Met Leu Arg Leu Ile Ala Leu Leu Val Cys Val Val Tyr Val Tyr Gly
1               5                   10                  15

Asp Asp Val Pro Tyr Ser Ser Asn Gln Gly Lys Cys Gly Gly His Asp
            20                  25                  30

Tyr Glu Lys Asp Gly Leu Cys Cys Ala Ser Cys His Pro Gly Phe Tyr
        35                  40                  45

Ala Ser Arg Leu Cys Gly Pro Gly Ser Asn Thr Val Cys Ser Pro Cys
    50                  55                  60

Glu Asp Gly Thr Phe Thr Ala Ser Thr Asn His Ala Pro Ala Cys Val
65                  70                  75                  80

Ser Cys Arg Gly Pro Cys Thr Gly His Leu Ser Glu Ser Gln Pro Cys
                85                  90                  95

Asp Arg Thr His Asp Arg Val Cys Asn Cys Ser Thr Gly Asn Tyr Cys
            100                 105                 110

Leu Leu Lys Gly Gln Asn Gly Cys Arg Ile Cys Ala Pro Gln Thr Lys
        115                 120                 125

Cys Pro Ala Gly Tyr Gly Val Ser Gly His Thr Arg Ala Gly Asp Thr
    130                 135                 140

Leu Cys Glu Lys Cys Pro Pro His Thr Tyr Ser Asp Ser Leu Ser Pro
145                 150                 155                 160

Thr Glu Arg Cys Gly Thr Ser Phe Asn Tyr Ile Ser Val Gly Phe Asn
                165                 170                 175

Leu Tyr Pro Val Asn Glu Thr Ser Cys Thr Thr Thr Ala Gly His Asn
            180                 185                 190
```

Glu Val Ile Lys Thr Lys Glu Phe Thr Val Thr Leu Asn Tyr Thr
195 200 205

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 227 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1 5 10 15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
20 25 30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
35 40 45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
50 55 60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65 70 75 80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
85 90 95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
100 105 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
115 120 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
130 135 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145 150 155 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe S r Asn Thr Thr Ser Ser Thr
165 170 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
180 185 190

Asn Ala Ser Arg Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
195 200 205

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
210 215 220

Gln His Thr
225

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 197 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

Met Val Ser Leu Pro Arg Leu Cys Ala Leu Trp Gly Cys Leu Leu Thr
1 5 10 15

Ala Val His Leu Gly Gln Cys Val Thr Cys Ser Asp Lys Gln Tyr Leu
20 25 30

His Asp Gly Gln Cys Cys Asp Leu Cys Gln Pro Gly Ser Arg Leu Thr
35 40 45

Ser His Cys Thr Ala Leu Glu Lys Thr Gln Cys His Pro Cys Asp Ser
50 55 60

Gly Glu Phe Ser Ala Gln Trp Asn Arg Glu Ile Arg Cys His Gln His
65 70 75 80

Arg His Cys Glu Pro Asn Gln Gly Leu Arg Val Lys Lys Glu Gly Thr
85 90 95

Ala Glu Ser Asp Thr Val Cys Thr Cys Lys Glu Gly Gln His Cys Thr
100 105 110

Ser Lys Asp Cys Glu Ala Cys Ala Gln His Thr Pr Cys Ile Pro Gly
115 120 125

Phe Gly Val Met Glu Met Ala Thr Glu Thr Thr Asp Thr Val Cys His
130 135 140

Pro Cys Pro Val Gly Phe Phe Ser Asn Gln Ser Ser Leu Phe Glu Lys
145 150 155 160

Cys Tyr Pro Trp Thr Ser Cys Glu Asp Lys Asn Leu Glu Val Leu Gln
165 170 175

Lys Gly Thr Ser Gln Thr Asn Val Ile Cys Gly Leu Lys Ser Arg Met
180 185 190

Arg Ala Leu Leu Val
195

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Phe Leu Asp Ile Ile
1 5 10 15

Glu Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp
20 25 30

Pro Glu Thr Gly Arg Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
35 40 45

Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
50 55 60

Cys Pro Asp Tyr Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65 70 75 80

Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Thr Val Lys Gln Glu
85 90 95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr
100 105 110

Leu Glu Leu Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Leu
115 120 125

Gly Val Leu Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg
130 135 140

Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145 150 155 160

Arg Lys His Thr Asn Cys Ser Ser Leu Gly Leu Leu Leu Ile Gln Lys
165 170 175

Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
180 185 190

Gln Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
195 200 205

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 224 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

Met Gly Ala Gly Ala Thr Gly Arg Ala Met Asp Gly Pro Arg Leu Leu
1 5 10 15

Leu Leu Leu Leu Leu Gly Val Ser Leu Gly Gly Ala Lys Glu Ala Cys
20 25 30

Pro Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys Lys Ala Cys Asn
35 40 45

Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala Asn Gln Thr Val Cys
50 55 60

Glu Pro Cys Leu Asp Ser Val Thr Phe Ser Asp Val Val Ser Ala Thr
65 70 75 80

Glu Pro Cys Lys Pro Cys Thr Glu Cys Val Gly Leu Gln Ser Met Ser
85 90 95

Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg Cys Ala Tyr Gly
100 105 110

Tyr Tyr Gln Asp Glu Thr Thr Gly Arg Cys Glu Ala Cys Arg Val Cys
115 120 125

Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln Asn Thr
130 135 140

Val Cys Glu Glu Cys Pro Asp Gly Thr Tyr Ser Asp Glu Ala Asn His
145 150 155 160

Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu Arg Gln
165 170 175

Leu Arg Glu Cys Thr Arg Trp Ala Asp Ala Glu Cys Glu Glu Ile Pro
180 185 190

Gly Arg Trp Ile Thr Arg Ser Thr Pro Pro Glu Gly Ser Asp Ser Thr
195 200 205

Ala Pro Ser Thr Gln Glu Pro Glu Ala Pro Pro Glu Gln Asp Leu Ile
210 215 220

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 205 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

Met Tyr Val Trp Val Gln Gln Pro Thr Ala Phe Leu Leu Leu Gly Leu
1 5 10 15

Ser Leu Gly Val Thr Val Lys Leu Asn Cys Val Lys Asp Thr Tyr Pro
20 25 30

Ser Gly His Lys Cys Cys Arg Glu Cys Gln Pro Gly His Gly Met Val
35 40 45

Ser Arg Cys Asp His Thr Arg Asp Thr Val Cys His Pro Cys Glu Pro
50 55 60

Gly Phe Tyr Asn Glu Ala Val Asn Tyr Asp Thr Cys Lys Gln Cys Thr
65 70 75 80

Gln Cys Asn His Arg Ser Gly Ser Glu Leu Lys Gln Asn Cys Thr Pro
85 90 95

Thr Glu Asp Thr Val Cys Gln Cys Arg Pro Gly Thr Gln Pro Arg Gln
100 105 110

Asp Ser Ser His Lys Leu Gly Val Asp Cys Val Pro Cys Pro Pro Gly
115 120 125

His Phe Ser Pro Gly Ser Asn Gln Ala Cys Lys Pro Trp Thr Asn Cys
130 135 140

Thr Leu Ser Gly Lys Gln Ile Arg His Pro Ala Ser Asn Ser Leu Asp
145 150 155 160

Thr Val Cys Glu Asp Arg Ser Leu Leu Ala Thr Leu Leu Trp Glu Thr
165 170 175

Gln Arg Thr Thr Phe Arg Pro Thr Thr Val Pro Ser Thr Thr Val Trp
180 185 190

Pro Arg Thr Ser Gln Leu Pro Ser Thr Pro Thr Leu Val
195 200 205

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

Met Gly Asn Asn Cys Tyr Asn Val Val Val Ile Val Leu Leu Leu Val
1 5 10 15

```
Gly Cys Glu Lys Val Gly Ala Val Gln Asn Ser Cys Asp Asn Cys Gln
            20                  25                  30

Pro Gly Thr Phe Cys Arg Lys Tyr Asn Pro Val Cys Lys Ser Cys Pro
        35                  40                  45

Pro Ser Thr Phe Ser Ser Ile Gly Gly Gln Pro Asn Cys Asn Ile Cys
    50                  55                  60

Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lys Phe Cys Ser Ser Thr
65                  70                  75                  80

His Asn Ala Glu Cys Glu Cys Ile Glu Gly Phe His Cys Leu Gly Pro
                85                  90                  95

Gln Cys Thr Arg Cys Glu Lys Asp Cys Arg Pro Gly Gln Glu Leu Thr
            100                 105                 110

Lys Gln Gly Cys Lys Thr Cys Ser Leu Gly Thr Phe Asn Asp Gln Asn
        115                 120                 125

Gly Thr Gly Val Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Arg
    130                 135                 140

Ser Val Leu Lys Thr Gly Thr Thr Glu Lys Asp Val Val Cys Gly Pro
145                 150                 155                 160

Pro Val Val Ser Phe Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Glu
                165                 170                 175

Gly Gly Pro Gly Gly His Ser Leu Gln Val Leu Thr Leu Phe Leu
            180                 185                 190
```

(2) INFORMATION FOR SEQ ID NO:137:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

```
TATGGATGAA GAAACTTCTC ATCAGCTGCT GTGTGATAAA TGTCCGCCGG GTAC          54
```

(2) INFORMATION FOR SEQ ID NO:138:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 380 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

```
Glu Thr Leu Pro Pro Lys Tyr Leu His Tyr Asp Pro Glu Thr Gly His
1               5                   10                  15

Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr Tyr Leu Lys Gln His
            20                  25                  30

Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro Cys Pro Asp His Ser
        35                  40                  45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Val Tyr Cys Ser Pro
    50                  55                  60

Val Cys Lys Glu Leu Gln Ser Val Lys Gln Glu Cys Asn Arg Thr His
65                  70                  75                  80

Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr Leu Glu Ile Glu Phe
                85                  90                  95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Ser Gly Val Val Gln Ala
            100                 105                 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Lys Cys Pro Asp Gly Phe
        115                 120                 125

Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys Ile Lys His Thr Asn
    130                 135                 140

Cys Ser Thr Phe Gly Leu Leu Leu Ile Gln Lys Gly Asn Ala Thr His
145                 150                 155                 160

Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr Gln Lys Cys Gly Ile
                165                 170                 175

Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr
            180                 185                 190
```

```
Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp Ser Leu Pro Gly
        195                 200                 205

Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Arg His Ser
    210                 215                 220

Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn
225                 230                 235                 240

Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys
                245                 250                 255

Glu Ser Ser Val Gln Arg His Leu Gly His Ser Asn Leu Thr Thr Glu
            260                 265                 270

Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly Lys Lys Ile Ser Pro
        275                 280                 285

Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Ser Ser Glu Gln Leu
    290                 295                 300

Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr
305                 310                 315                 320

Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys Thr Ser His Phe
                325                 330                 335

Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Met Arg Phe Leu His
            340                 345                 350

Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile
        355                 360                 365

Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
    370                 375                 380
```

(2) INFORMATION FOR SEQ ID NO:139:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 380 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

```
Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
1               5                   10                  15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
            20                  25                  30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
        35                  40                  45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
    50                  55                  60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65                  70                  75                  80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
                85                  90                  95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
            100                 105                 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
        115                 120                 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
    130                 135                 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145                 150                 155                 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile
                165                 170                 175

Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr
            180                 185                 190

Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly
        195                 200                 205

Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser
    210                 215                 220

Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn
225                 230                 235                 240

Lys Ala Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys
                245                 250                 255
```

```
Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu
            260                 265                 270

Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala
        275                 280                 285

Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile
    290                 295                 300

Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr
305                 310                 315                 320

Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe
                325                 330                 335

Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His
            340                 345                 350

Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile
        355                 360                 365

Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
    370                 375                 380
```

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

TGGACCACCC AGAAGTACCT TCATTATGAC 30

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

GTCATAATGA AGGTACTTCT GGGTGGTCCA 30

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

GGACCACCCA GCTTCATTAT GACGAAGAAA C 31

(2) INFORMATION FOR SEQ ID NO:143:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

GTTTCTTCGT CATAATGAAG CTGGGTGGTC C 31

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

GTGGACCACC CAGGACGAAG AAACCTCTC 29

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

GAGAGGTTTC TTCGTCCTGG GTGGTCCAC 29

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

CGTTTCCTCC AAAGTTCCTT CATTATGAC 29

(2) INFORMATION FOR SEQ ID NO:147:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

GTCATAATGA AGGAACTTTG GAGGAAACG 29

(2) INFORMATION FOR SEQ ID NO:148:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

GGAAACGTTT CCTGCAAAGT ACCTTCATTA TG 32

(2) INFORMATION FOR SEQ ID NO:149:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

CATAATGAAG GTACTTTGCA GGAAACGTTT CC 32

(2) INFORMATION FOR SEQ ID NO:150:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

CACGCAAAAG TCGGGAATAG ATGTCAC 27

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

GTGACATCTA TTCCCGACTT TTGCGTG 27

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

CACCCTGTCG GAAGAGGCCT TCTTC 25

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

GAAGAAGGCC TCTTCCGACA GGGTG 25

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

TGACCTCTCG GAAAGCAGCG TGCA 24

(2) INFORMATION FOR SEQ ID NO:155:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

TGCACGCTGC TTTCCGAGAG GTCA 24

(2) INFORMATION FOR SEQ ID NO:156:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

CCTCGAAATC GAGCGAGCAG CTCC 24

(2) INFORMATION FOR SEQ ID NO:157:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

CGATTTCGAG GTCTTTCTCG TTCTC 25

(2) INFORMATION FOR SEQ ID NO:158:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

CCGTGAAAAT AAGCTCGTTA TAACTAGGAA TGG 33

(2) INFORMATION FOR SEQ ID NO:159:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

CCATTCCTAG TTATAACGAG CTTATTTTCA CGG 33

(2) INFORMATION FOR SEQ ID NO:160:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG 38

(2) INFORMATION FOR SEQ ID NO:161:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

CCTCTCTCGA GTCAGGTGAC ATCTATTCCA CACTTTTGCG TGGC 44

(2) INFORMATION FOR SEQ ID NO:162:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG 38

(2) INFORMATION FOR SEQ ID NO:163:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

CCTCTCTCGA GTCAAGGAAC AGCAAACCTG AAGAAGGC 38

(2) INFORMATION FOR SEQ ID NO:164:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG 38

(2) INFORMATION FOR SEQ ID NO:165:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

CCTCTCTCGA GTCACTCTGT GGTGAGGTTC GAGTGGCC 38

(2) INFORMATION FOR SEQ ID NO:166:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG 38

(2) INFORMATION FOR SEQ ID NO:167:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

CCTCTCTCGA GTCAGGATGT TTTCAAGTGC TTGAGGGC 38

(2) INFORMATION FOR SEQ ID NO:168:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: prot in

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

Met Lys His His His His His His His Ala Ser Val Asn Ala Leu Glu
1 5 10 15

(Rel 66-12/95 Pub 6615) FORM 13-27 13-205

Applicant's or agent's file reference number	A-378-CIP2	International application No.	

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 15,16,45,60,64,67,100 line Too many to list

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, MD 20852

Date of deposit	Accession Number
12/27/95 and 7/24/96	69969,69970,69971,98112 and 98113

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications, e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer Elnora Rivers, Paralegal Specialist, IAPD - PCT Operations, (703) 305-3678	Authorized officer

Form PCT/RO/134 (July 1992)

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a polypeptide comprising at least one of the biological activities of OPG wherein the nucleic acid is selected from the group consisting of:

a) the nucleic acids shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID NO:124) or complementary strands thereof;

b) nucleic acids which hybridize under stringent conditions with the polypeptide-encoding regions as shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122) and 9C-9D (SEQ ID NO:124);

c) nucleic acids which hybridize under stringent conditions with nucleotides 148 through 337 inclusive as shown in Figure 1A; and

d) nucleic acid which are degenerate to the nucleic acids of (a), (b) and (c).

2. The nucleic acid of Claim 1 which is cDNA, genomic DNA, synthetic DNA or RNA.

3. A polypeptide encoded by the nucleic acid of Claim 1.

4. The nucleic acid of Claim 1 including one or more codons preferred for Escherichia coli expression.

5. The nucleic acid of Claim 1 having a detectable label attached thereto.

6. The nucleic acid of Claim 1 comprising the polypeptide-encoding region of Figure 2B-2C (SEQ ID NO:120), Figure 9A-9B (SEQ ID NO:122) or Figure 9C-9D (SEQ ID NO:124).

7. The nucleic acid of Claim 6 having the sequence as shown in Figure 9C-D (SEQ ID NO:124) from nucleotides 158-1297.

8. An expression vector comprising the nucleic acid of Claim 1.

9. The expression vector of Claim 8 wherein the nucleic acid comprises the polypeptide - encoding region as shown in Figure 9C-9D (SEQ ID NO:124).

10. A host cell transformed or transfected with the expression vector of Claim 8.

11. The host cell of Claim 10 which is a eucaryotic cell.

12. The host cell of Claim 11 which is selected from the group consisting of CHO, COS, 293, 3T3, CV-1 and BHK cells.

13. The host cell of Claim 10 which is a procaryotic cell.

14. The host cell of Claim 13 which is Escherichia coli.

15. A transgenic mammal comprising the expression vector of Claim 8.

16. The transgenic mammal of Claim 15 which is a rodent.

17. The transgenic mammal of Claim 16 which is a mouse.

18. A process for the production of OPG comprising:

growing under suitable nutrient conditions host cells transformed or transfected with the nucleic acid of Claim 1; and

isolating the polypeptide products of the expression of the nucleic acids.

19. A purifed and isolated polypeptide comprising OPG.

20. The polypeptide of Claim 19 which is mammalian OPG.

21. The polypeptide of Claim 20 which is human OPG.

22. The polypeptide of Claim 19 which is substantially free of other human proteins.

23. The polypeptide of Claim 21 having the amino acid sequence as shown in Figure 2B-2C (SEQ ID NO:121), Figure 9A-9B (SEQ ID NO:123), or Figure 9C-9D (SEQ ID NO:125) or a derivative thereof.

24. The polypeptide of Claim 23 having the amino acid sequence as shown in Figure 9C-9D (SEQ ID NO:125) from residues 22-401 inclusive.

25. The polypeptide of Claim 23 having the amino acid sequence as shown in Figure 9C-9D (SEQ ID NO:125) from residues 32-401 inclusive.

26. The polypeptide of Claim 19 which is characterized by being a product of expression of an exogenous DNA sequence.

27. The polypeptide of Claim 26 wherein the DNA is cDNA, genomic DNA or synthetic DNA.

28. The polypeptide of Claim 19 which has been modified with a water-soluble polymer.

29. The polypeptide of Claim 28 wherein the water soluble polymer is polyethylene glycol.

30. A polypeptide comprising:

an amino acid sequence of at least about 164 amino acids comprising four cysteine-rich domains characteristic of the cysteine rich domains of tumor necrosis factor receptor extracellular regions; and

an activity of increasing bone density.

31. A polypeptide comprising the amino acid sequence as shown in Figure 2B-2C (SEQ ID NO:121), Figure 9A-9B (SEQ ID NO:123) or Figure 9C-9D (SEQ ID NO:125) having an amino terminus at residue 22, and wherein from 1 to 216 amino acids are deleted from the carboxy terminus.

32. The polypeptide of Claim 31 comprising the amino acid sequence from residues 22-185, 22-189, 22-194, or 22-201 inclusive.

33. The polypeptide of Claim 32 further comprising an Fc region of human IgG1 extending from the carboxy terminus.

34. A polypeptide comprising the amino acid sequence as shown in Figure 2B-2C (SEQ ID NO:121), Figure 9A-9B (SEQ ID NO:123) or Figure 9C-9D (SEQ ID NO:125) having an amino terminus at residue 22, wherein from 1 to 10 amino acids are deleted from the amino terminus and, optionally, from 1 to 216 amino acids are deleted from the carboxy terminus.

35. The polypeptide of Claim 34 comprising the amino acid sequence from residues 27-185, 27-189, 27-194, 27-401, or 32-401 inclusive.

36. The polypeptide of Claim 35 further comprising an Fc region of human IgG1 extending from the carboxy terminus.

37. A polypeptide selected from the group consisting of:

huOPG [22-201]-Fc
huOPG [22-401]-Fc
huOPG [22-180]-Fc
huOPG met [22-401]-Fc
huOPG Fc-met [22-401]
huOPG met [22-185]
huOPG met [22-189]

huOPG met [22-194]
huOPG met [27-185]
huOPG met [27-189]
huOPG met [27-194]
huOPG met [32-401]
huOPG met-lys[22-401]
huOPG met [22-401]
huOPG met [22-401]-Fc (P25A)
huOPG met [22-401] (P25A)
huOPG met [22-401] (P26A)
huOPG met [22-401] (P26D)
huOPG met [22-194] (P25A)
huOPG met [22-194] (P26A)
huOPG met met-(lys)$_3$ [22-401]
huOPG met met-arg-gly-ser-(his)$_6$ [22-401]

38. A nucleic acid encoding the polypeptide of Claim 37.

39. An antibody or fragment thereof which specifically binds to OPG.

40. The antibody of Claim 39 which is a monoclonal antibody.

41. A method for detecting the presence of OPG in a biological sample comprising:

incubating the sample with the antibody of Claim 39 under conditions that allow binding of the antibody to OPG; and

detecting the bound antibody.

42. A method to assess the ability of a candidate substance to bind to OPG comprising:
incubating OPG with the candidate substance under conditions that allow binding; and
measuring the bound substance.

43. A method of regulating the levels of OPG in an animal comprising modifying the animal with a nucleic acid encoding OPG.

44. The method of Claim 43 wherein the nucleic acid promotes an increase in the tissue level of OPG.

45. The method of Claim 44 wherein the animal is a human.

46. A pharmaceutical composition comprising a therapeutically effective amount of OPG in a pharmaceutically acceptable carrier, adjuvant, solubilizer, stabilizer and/or anti-oxidant.

47. The composition of Claim 46 wherein the OPG is human OPG.

48. The composition of Claim 47 wherein the OPG has the amino acid sequence as shown in Figure 9B.

49. A method of treating a bone disorder comprising administering a therapeutically effective amount of the polypeptide of Claim 19.

50. The method of Claim 49 wherein the polypeptide is human OPG.

51. The method of Claim 49 wherein the bone disorder is excessive bone loss.

52. The method of Claim 51 wherein the bone disorder is selected from the group consisting of osteoporosis, Paget's disease of bone, hypercalcemia, hyperparathyroidism, steroid-induced osteopenia, bone loss due to rheumatoid arthritis, bone loss due to osteomyelitis, osteolytic metastasis, and periodontal bone loss.

53. The method of Claim 49 further comprising administering a therapeutically effective amount of a substances selected from the group consisting of bone morphogenic proteins BMP-1 through BMP-12, TGF-β family members, IL-1 inhibitors, TNFα inhibitors, parathyroid hormone and analogs thereof, parathyroid hormone related protein and analogs thereof, E series prostaglandins, bisphosphonates, and bone-enhancing minerals.

54. An osteoprotegerin multimer consisting of osteoprotegerin monomers.

55. The multimer of Claim 54 which is a dimer.

56. The multimer of Claim 54 formed by interchain disulfide bonds.

57. The multimer of Claim 54 formed by association Fc regions derived from human IgG1.

58. The multimer of Claim 54 which is essentially free of osteoprotegerin monomers and inactive multimers.

59. The multimer of Claim 54 wherein the monomers comprise the amino acid sequence as shown in Figure 9C-9D (SEQ ID NO:125) from residues 22-401, or a derivative thereof.

60. The multimer of Claim 54 wherein the monomers comprise the amino acid sequence as shown in Figure 9C-9D (SEQ ID NO:125) from residues 22-194.

FIG.1A

```
                148       178       208       238       268       298
FRI-1           ALLVFLDIIEWTTQETFPPKYLHYDPETGRQLLCDKCAPGTYLKQHCTVRRKTLCVPCPD
                                              |: | ||:||  | ||  |:| :| |
SW:TNR2_HUMAN   HALPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCED
                       30        40        50        60        70        80

                328
FRI-1           YSYTDSWHTS
                 :||: |:
SW:TNR2_HUMAN   STYTQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPL
                       90       100       110       120       130       140
```

FIG.1B

```
FRI-1          69 YLHYDPETGRQLLCDKCAPGTYLKQHC.TVRRKTLCV.PCPDY.SYTDSW
                  | |. ...  | .| || :|  .|   : | |  || :   |.| .
TNFR profile    6 YHYYDQNGRMCEECHMCQPGHFLVKHCKQPKRDTVCHKPCEPGVTYTDDW

FRI-1         116 H
                  |
TNFR profile   56 H                                     Z Score = 8.29
```

FIG. 1C

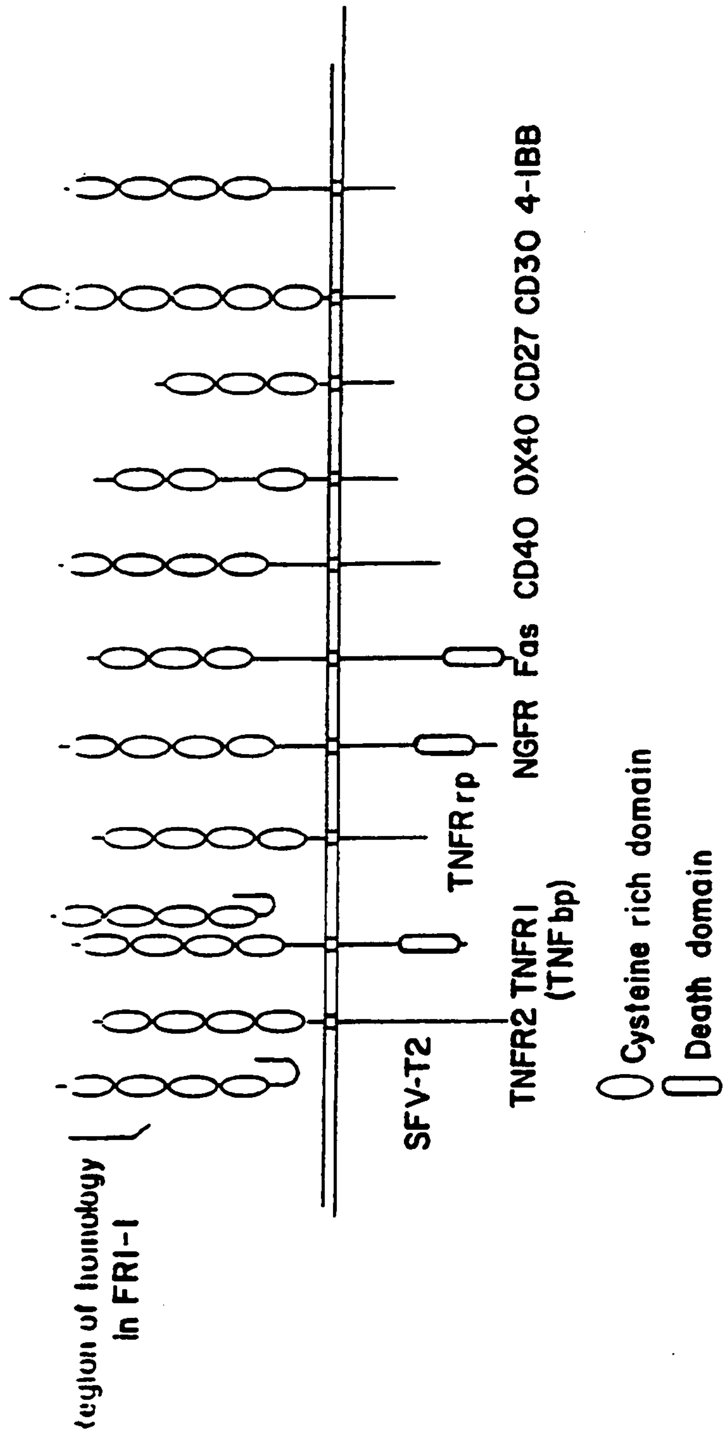

Region of homology
in FRI-I
SFV-T2
TNFR2
TNFR1
(TNFbp)
TNFRrp
NGFR
Fas
CD40
OX40
CD27
CD30
4-1BB
Cysteine rich domain
Death domain

FIG.2A

FIG.2B

```
10                             30                             50
ATCAAAGGCAGGGCATACTTCCTGTTGCCCAGACCTTATATAAAACGTCATGTTCGCCTG
70                             90                             110
GGCAGCAGAGAAGCACCTAGCACTGGCCCAGCGGCTGCCGCCTGAGGTTTCCAGAGGACC
130                            150                            170
ACAATGAACAAGTGGCTGTGCTGTGCACTCCTGGTGTTCTTGGACATCATTGAATGGACA
    M  N  K  W  L  C  C  A  L  L  V  F  L  D  I  I  E  W  T
190                            210                            230
ACCCAGGAAACCTTTCCTCCAAAATACTTGCATTATGACCCAGAAACCGGACGTCAGCTC
T  Q  E  T  F  P  P  K  Y  L  H  Y  D  P  E  T  G  R  Q  L
250                            270                            290
TTGTGTGACAAATGTGCTCCTGGCACCTACCTAAAACAGCACTGCACAGTCAGGAGGAAG
L  C  D  K  C  A  P  G  T  Y  L  K  Q  H  C  T  V  R  R  K
310                            330                            350
ACACTGTGTGTCCCTTGCCCTGACTACTCTTATACAGACAGCTGGCACACGAGTGATGAA
T  L  C  V  P  C  P  D  Y  S  Y  T  D  S  W  H  T  S  D  E
370                            390                            410
TGCGTGTACTGCAGCCCCGTGTGCAAGGAACTGCAGACCGTGAAACAGGAGTGCAACCGC
C  V  Y  C  S  P  V  C  K  E  L  Q  T  V  K  Q  E  C  N  R
430                            450                            470
ACCCACAACCGAGTGTGCGAATGTGAGGAAGGGCGCTACCTGGAGCTCGAATTCTGCTTG
T  H  N  R  V  C  E  C  E  E  G  R  Y  L  E  L  E  F  C  L
490                            510                            530
AAGCACCGGAGCTGTCCCCCAGGCTTGGGTGTGCTGCAGGCTGGGACCCCAGAGCGAAAC
K  H  R  S  C  P  P  G  L  G  V  L  Q  A  G  T  P  E  R  N
550                            570                            590
ACGGTTTGCAAAAGATGTCCGGATGGGTTCTTCTCAGGTGAGACGTCATCGAAAGCACCC
T  V  C  K  R  C  P  D  G  F  F  S  G  E  T  S  S  K  A  P
610                            630                            650
TGTAGGAAACACACCAACTGCAGCTCACTTGGCCTCCTGCTAATTCAGAAAGGAAATGCA
C  R  K  H  T  N  C  S  S  L  G  L  L  L  I  Q  K  G  N  A
670                            690                            710
ACACATGACAATGTATGTTCCGGAAACAGAGAAGCAACTCAAAATTGTGGAATAGATGTC
T  H  D  N  V  C  S  G  N  R  E  A  T  Q  N  C  G  I  D  V
730                            750                            770
ACCCTGTGCGAAGAGGCATTCTTCAGGTTTGCTGTGCCTACCAAGATTATACCGAATTGG
T  L  C  E  E  A  F  F  R  F  A  V  P  T  K  I  I  P  N  W
790                            810                            830
CTGAGTGTTCTGGTGGACAGTTTGCCTGGGACCAAAGTGAATGCAGAGAGTGTAGAGAGG
L  S  V  L  V  D  S  L  P  G  T  K  V  N  A  E  S  V  E  R
850                            870                            890
ATAAAACGGAGACACAGCTCGCAAGAGCAAACTTTCCAGCTACTTAAGCTGTGGAAGCAT
I  K  R  R  H  S  S  Q  E  Q  T  F  Q  L  L  K  L  W  K  H
910                            930                            950
CAAAACAGAGACCAGGAAATGGTGAAGAAGATCATCCAAGACATTGACCTCTGTGAAAGC
Q  N  R  D  Q  E  M  V  K  K  I  I  Q  D  I  D  L  C  E  S
970                            990                            1010
AGTGTGCAACGGCATATCGGCCACGCGAACCTCACCACAGAGCAGCTCCGCATCTTGATG
S  V  Q  R  H  I  G  H  A  N  L  T  T  E  Q  L  R  I  L  M
```

FIG.2C

```
       1030                      1050                      1070
GAGAGCTTGCCTGGGAAGAAGATCAGCCCAGACGAGATTGAGAGAACGAGAAAGACCTGC
E  S  L  P  G  K  K  I  S  P  D  E  I  E  R  T  R  K  T  C
       1090                      1110                      1130
AAACCCAGCGAGCAGCTCCTGAAGCTACTGAGCTTGTGGAGGATCAAAAATGGAGACCAA
K  P  S  E  Q  L  L  K  L  L  S  L  W  R  I  K  N  G  D  Q
       1150                      1170                      1190
GACACCTTGAAGGGCCTGATGTACGCACTCAAGCACTTGAAAGCATACCACTTTCCCAAA
D  T  L  K  G  L  M  Y  A  L  K  H  L  K  A  Y  H  F  P  K
       1210                      1230                      1250
ACCGTCACCCACAGTCTGAGGAAGACCATCAGGTTCTTGCACAGCTTCACCATGTACCGA
T  V  T  H  S  L  R  K  T  I  R  F  L  H  S  F  T  M  Y  R
       1270                      1290                      1310
TTGTATCAGAAACTCTTTCTAGAAATGATAGGGAATCAGGTTCAATCAGTGAAGATAAGC
L  Y  Q  K  L  F  L  E  M  I  G  N  Q  V  Q  S  V  K  I  S
       1330                      1350                      1370
TGCTTATAGTTAGGAATGGTCACTGGGCTGTTTCTTCAGGATGGGCCAACACTGATGGAG
C  L
       1390                      1410                      1430
CAGATGGCTGCTTCTCCGGCTCTTGAAATGGCAGTTGATTCCTTTCTCATCAGTTGGTGG
       1450                      1470                      1490
GAATGAAGATCCTCCAGCCCAACACACACACTGGGGAGTCTGAGTCAGGAGAGTGAGGCA
       1510                      1530                      1550
GGCTATTTGATAATTGTGCAAAGCTGCCAGGTGTACACCTAGAAAGTCAAGCACCCTGAG
       1570                      1590                      1610
AAAGAGGATATTTTTATAACCTCAAACATAGGCCCTTTCCTTCCTCTCCTTATGGATGAG
       1630                      1650                      1670
TACTCAGAAGGCTTCTACTATCTTCTGTGTCATCCCTAGATGAAGGCCTCTTTTATTTAT
       1690                      1710                      1730
TTTTTTATTCTTTTTTTCGGAGCTGGGGACCGAACCCAGGGCCTTGCGCTTGCGAGGCAA
       1750                      1770                      1790
GTGCTCTACCACTGAGCTAAATCTCCAACCCCTGAAGGCCTCTTTCTTTCTGCCTCTGAT
       1810                      1830                      1850
AGTCTATGACATTCTTTTTTCTACAATTCGTATCAGGTGCACGAGCCTTATCCCATTTGT
       1870                      1890                      1910
AGGTTTCTAGGCAAGTTGACCGTTAGCTATTTTTCCCTCTGAAGATTTGATTCGAGTTGC
       1930                      1950                      1970
AGACTTGGCTAGACAAGCAGGGGTAGGTTATGGTAGTTTATTTAACAGACTGCCACCAGG
       1990                      2010                      2030
AGTCCAGTGTTTCTTGTTCCTCTGTAGTTGTACCTAAGCTGACTCCAAGTACATTTAGTA
       2050                      2070                      2090
TGAAAAATAATCAACAAATTTTATTCCTTCTATCAACATTGGCTAGCTTTGTTTCAGGGC
       2110                      2130                      2150
ACTAAAAGAAACTACTATATGGAGAAAGAATTGATATTGCCCCCAACGTTCAACAACCCA
       2170                      2190                      2210
ATAGTTTATCCAGCTGTCATGCCTGGTTCAGTGTCTACTGACTATGCGCCCTCTTATTAC
       2230                      2250                      2270
TGCATGCAGTAATTCAACTGGAAATAGTAATAATAATAATAGAAATAAAATCTAGACTCC
       2290                      2310                      2330
ATTGGATCTCTCTGAATATGGGAATATCTAACTTAAGAAGCTTTGAGATTTCAGTTGTGT
       2350                      2370                      2390
TAAAGGCTTTTATTAAAAAGCTGATGCTCTTCTGTAAAAGTTACTAATATATCTGTAAGA
       2410                      2430
CTATTACAGTATTGCTATTTATATCCATCCAG
```

FIG.2D

```
  fas.frg  M L G I W T - - - - L L P L V L T S - V A R L S S K S V N A Q V T D I N S K G L E L R K T V T T V E   45
tnfr1.frg  - M G L S T V P D L L L P L V L L E L L V G I Y P S G V I G L V P H - - - - - L G D R E K R D S V C   44
sfv-t2.frg - - - - - - - - - - - - - - - - - - - - - - - - - M L R L I A L L V C V V Y V Y G D D V P Y S S N Q   25
tnfr2.frg  - - - - - - - - - - - M A P V A V W A A L A V G L E L W A A A H A L P A Q V A F T P Y A P E P G S T   39
 cd40.frg  - - - - - - - - - - - - - - - - - - - - - - M V S L P R L C A L W G C L L T A V H L G Q C V T C S D   28
osteo.frg  - - - - - - - - - - - - - - - - - - - - - - - M N K W L C C A L L V F L D I I E W T T Q E T F P P   26
 ngfr.frg  - - - - - - - - - - - - - - - M G A G A T G R A M D G P R L L L L L L L G V S L G - G A K E A C P T   34
 ox40.frg  - - - - - - - - - - - - - - - - - - - - - - M Y V W V Q Q P T A F L L L G L S L G V T V K L N C V K   28
 41bb.frg  - - - - - - - - - - - - - - - - - - - - - - - - - M G N N C Y N V V V I V L L L V G C E K V G A V Q   25

  fas.frg  T Q N L E G L H H D G Q F C H K P C P P G E R K A R D C T V N G D E P D C V P C Q E G K E Y T D K A   95
tnfr1.frg  P Q G K Y I H P Q N N S I C C T K C H K G T Y L Y N D C P G P G Q D T D C R E C E S G S F T A S E N   94
sfv-t2.frg G K C G G H D Y E K D G L C C A S C H P G F Y A S R L C G P G S N T V C S P C - E D G T F T A S T N   74
tnfr2.frg  C R L R E Y Y D Q T A Q M C C S K C S P G Q H A K V F C T K T S D T V C D S C - E D S T Y T Q L W N   88
 cd40.frg  K Q Y L H D G Q C - - - - - C D L C Q P G S R L T S H C T A L E K T Q C H P C - D S G E F S A Q W N   72
osteo.frg  K Y L H Y D P E T G R Q L L C D K C A P G T Y L K Q H C T V R R K T L C V P C - P D Y S Y T D S W H   75
 ngfr.frg  G L Y T H S G E - - - - - C C K A C N L G E G V A Q P C G - A N Q T V C E P C L D S V T F S D V V S   78
 ox40.frg  D T Y P S G H K - - - - - C C R E C Q P G H G M V S R C D H T R D T V C H P C - E P G F Y N E A V N   72
 41bb.frg  N - - - - - - - - - - - - S C D N C Q P G T F C R K Y - - - - - - N P V C K S C P P S T F S S - - -   54

  fas.frg  H F - - - - - S S K C R R - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   103
tnfr1.frg  H L R H C L S C S K C R K E M G Q V E I S S C T V D R D T V C G C R K N Q Y R H Y W S E N L F Q C F   144
sfv-t2.frg H - - - A P A C V S C R G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   84
tnfr2.frg  W - - - V P E C L S C G S - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   98
 cd40.frg  R E I R C H Q H R H C E P - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   85
osteo.frg  - - - T S D E C V Y C S P - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   85
 ngfr.frg  A T E P C K P C T E C - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   89
 ox40.frg  - Y D T C K Q C T Q C N H - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   84
 41bb.frg  - - I G G Q P N C N I C R - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   65
```

FIG.2E

```
fas.frg    - C R L C D E G H G L E V E I N C T R T Q N T K C R C K P N F F C N S T V C E H C D P C T K C E H G   152
tnfr1.frg  N C S L C L N G - - - T V H L S C Q E K Q N T V C T C H A G F F L R E N E C V S C S N C K K S L E C   191
sfv-t2.frg - - - - - P C T G H L S E S Q P C D R T H D R V C N C S T G N Y C L L K G Q N G C R I C A P Q T K C   129
tnfr2.frg  - - - - - R C S S D Q V E T Q A C T R E Q N R I C T C R P G W Y C A L S K Q E G C R L C A P L R K C   143
cd40.frg   - - - - - - - - N Q G L R V K K E G T A E S D T V C T C K E G Q H C T S K D C E - - - A C A Q H T P C   125
osteo.frg  - - - - - V C K E L Q T V K Q E C N R T H N R V C E C E E G R Y - - - L E L E - - - F C L K H R S C   124
ngfr.frg   - - - - - V G L Q S M - - S A P C V E A D D A V C R C A Y G Y Y - - - - Q D E T T G R C E A C R V C   128
ox40.frg   - - - - - R S G S E L - - K Q N C T P T E D T V C Q C - - - - - - - - - - R P G T Q P R Q D S S H - -   116
41bb.frg   - - - - - V C A G Y F R F K K F C S S T H N A E C E C I E G F H C L G P Q C T R C - - - - - - E K D C   105

fas.frg    - - - - - I I K E C T L T S N T K C K E - - - - - - - E G S R S N L - - - - G W L C L L L P I P L I   187
tnfr1.frg  - - - - - T K L C L P Q I E N V K G T E - - - - - - - D S G T T V L L P L V I F F G L C L L S L L F I   230
sfv-t2.frg P A G Y G V S - G H T R A G D T L C E K C P P H T Y S D S L S P T E R C G T S F N Y I S V G F N L Y   178
tnfr2.frg  R P G F G V A R P G T E T S D V V C K P C A P G T F S N T T S S T D I C R P H Q I C N V V A I P G N   193
cd40.frg   I P G F G V M E M A T E T T D T V C H P C P V G F F S N Q S S L F E K C Y P W T S C E D K N L E V L   175
osteo.frg  P P G L G V L Q A G T P E R N T V C K R C P D G F F S G E T S S K A P C R K H T N C S S L G L L L I   174
ngfr.frg   E A G S G L V F S C Q D K Q N T V C E E C P D G T Y S D E A N H V D P C L P C T V C E D T E R Q L R   178
ox40.frg   - - - - - - - - - - - - - K L G V D C V P C P P G H F S P G S N - - Q A C K P W T N C T L S G K Q I R   152
41bb.frg   R P G Q E L T K Q G - - - - - - - - C K T C S L G T F N D Q - N G T G V C R P W T N C S L D G R S V L   147

fas.frg    V W V - - - - - - - K R K E V Q K T C R K H R K E N Q G S H E S P T L N P E - - - - - - - - - - - - - T   219
tnfr1.frg  G L M Y R Y Q R W K S K L Y S I V C G K S T P E K E G E L E G T T T K P L A P N P S F S P T P G F T   280
sfv-t2.frg P V N - - - - - - - - - - - - - - - - - - E T S C T T T A - - - - - G H N E V I K T K E F T V T L N Y T   207
tnfr2.frg  A S R - - - - - - - - - - - - - - - - - - D A V C T S T S P T R S M A P G A V H L P Q P V S T R S Q H T   227
cd40.frg   Q K G - - - - - - - - - - - - - - - - - - - T S Q T N V I C G L K S R M R - - - - - - - - - - - - A L L V   197
osteo.frg  Q K G - - - - - - - - - - - - - - - - - - N A T H D N V C S G N R E A T Q N C G I D V T L C E E A F F R   208
ngfr.frg   E C T R W A D A E C E E I P G R - - W I T R S T - - P P E G S D S T A P S T Q E P E A P P E Q D L I   224
ox40.frg   H P A S N S V C E D R S L L A T L L W E T Q R T T F R P T T V P S T T V W P R T S Q L P S T P T L V   202
41bb.frg   K T G T T E K D V V C G P P V V S F S P S T T I S V T P E G G P G - - - - - - - G H S L Q V L T L F L   191
```

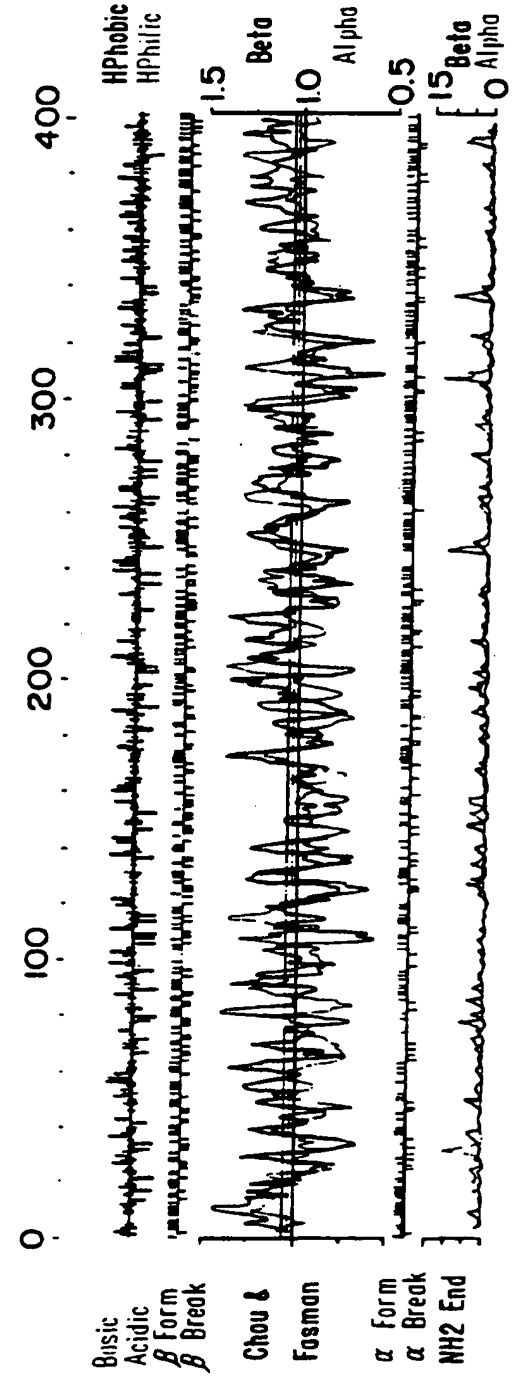


FIG.3A
FIG.3B
FIG.3C
FIG.3D
FIG.3E

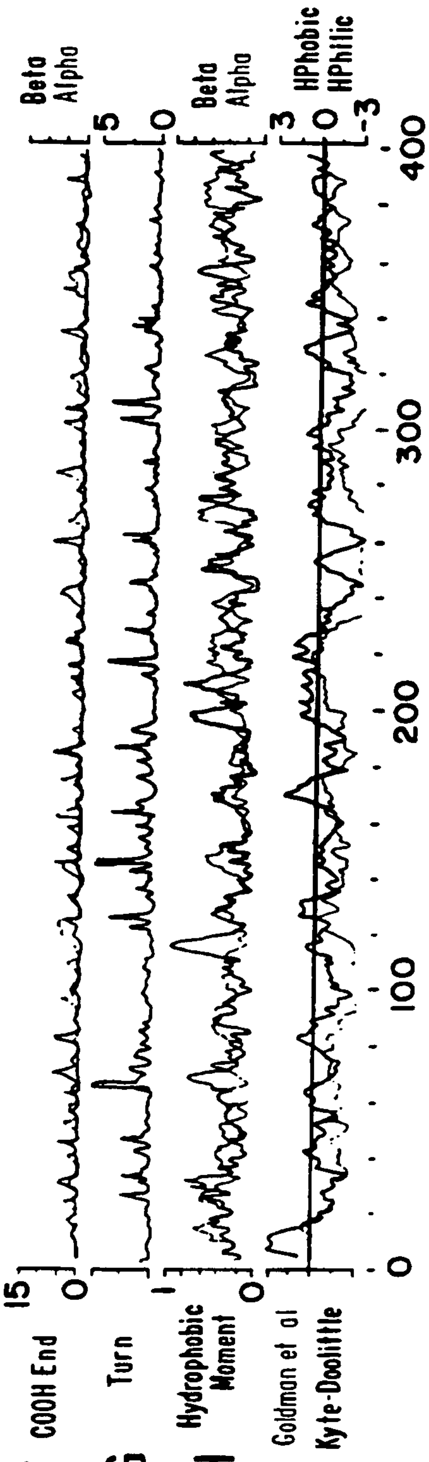


FIG.3F

FIG.3G

FIG.3H

FIG.3I

FIG.4A

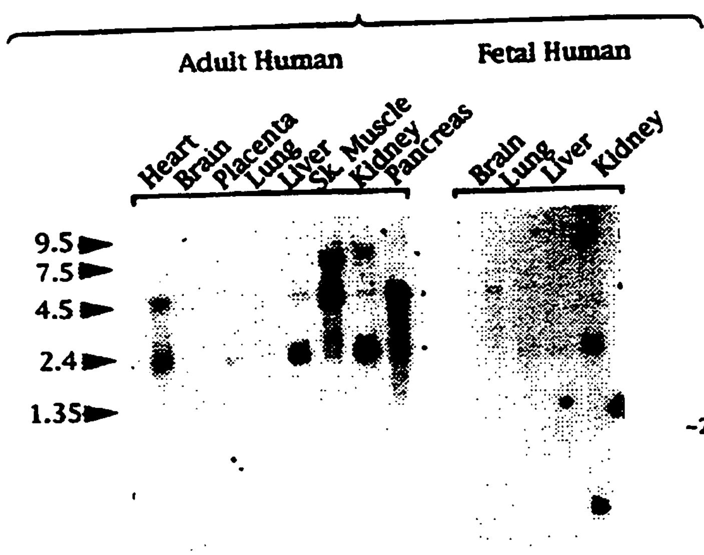

Adult Human
Fetal Human
Heart
Brain
Placenta
Lung
Liver
Sk. Muscle
Kidney
Pancreas
Brain
Lung
Liver
Kidney
9.5
7.5
4.5
2.4
1.35

FIG.4B

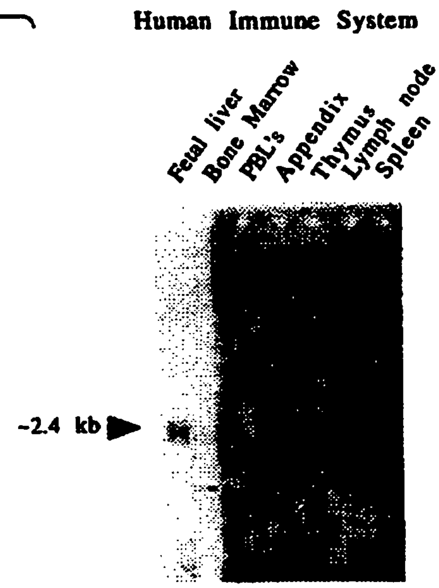

Human Immune System
Fetal liver
Bone Marrow
PBL's
Appendix
Thymus
Lymph node
Spleen
~2.4 kb

FIG.5

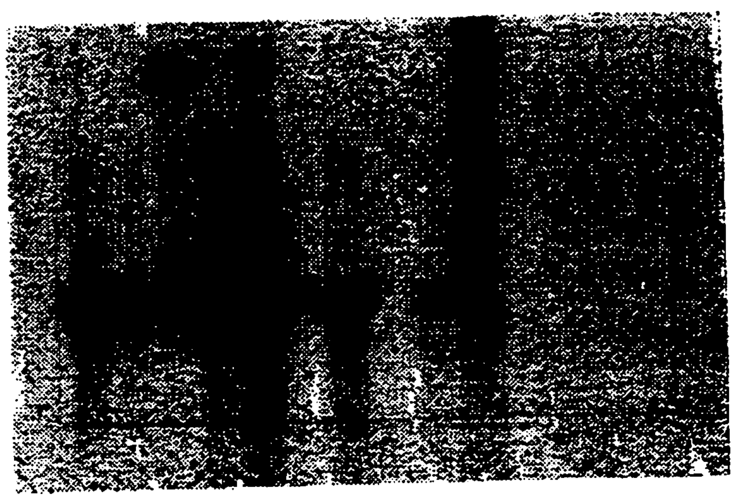

2 11 16 17 22 28 33 38 45
Kb
1 12 18 30
Transgenic Founders
Controls

FIG.6A

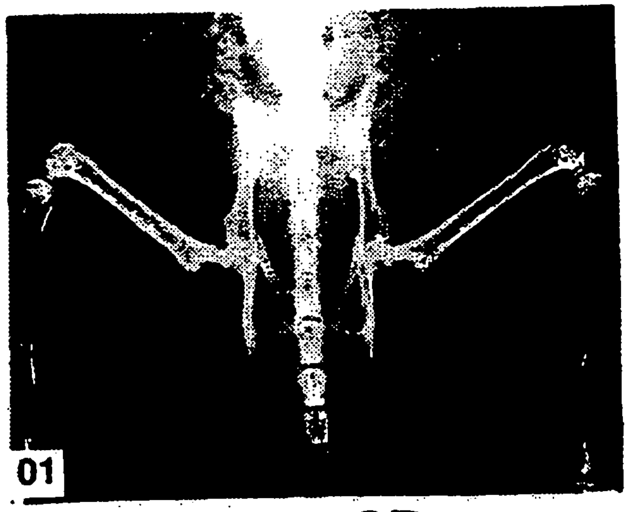


FIG.6B

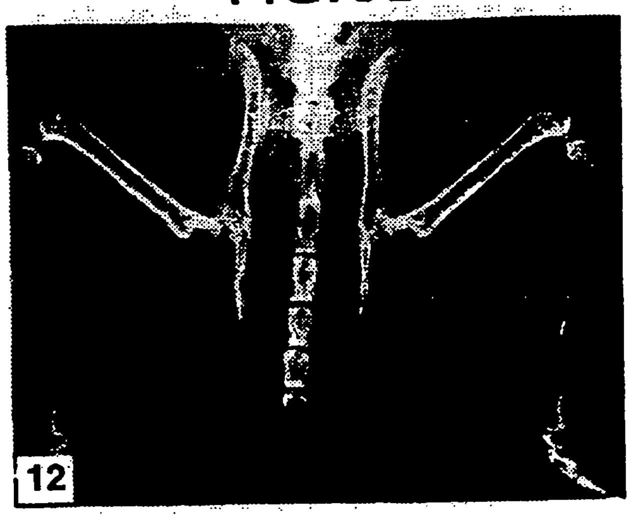


FIG.6C

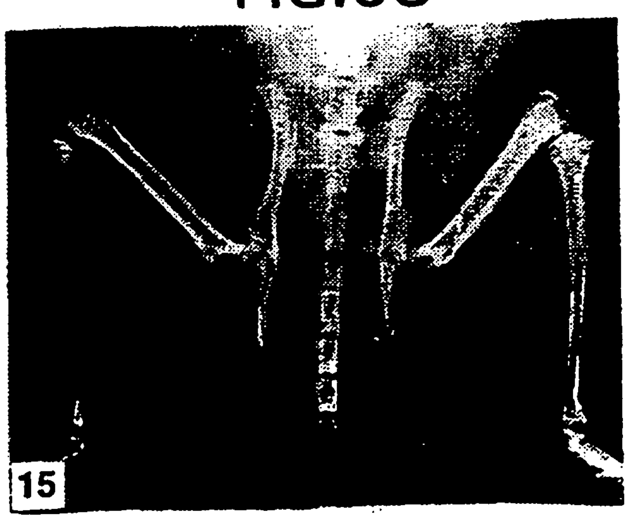


FIG.6D

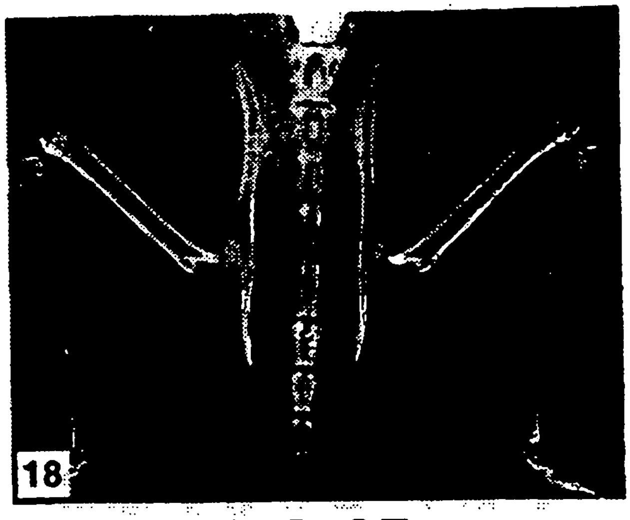


FIG.6E

FIG.6F

FIG.6G

FIG.6H

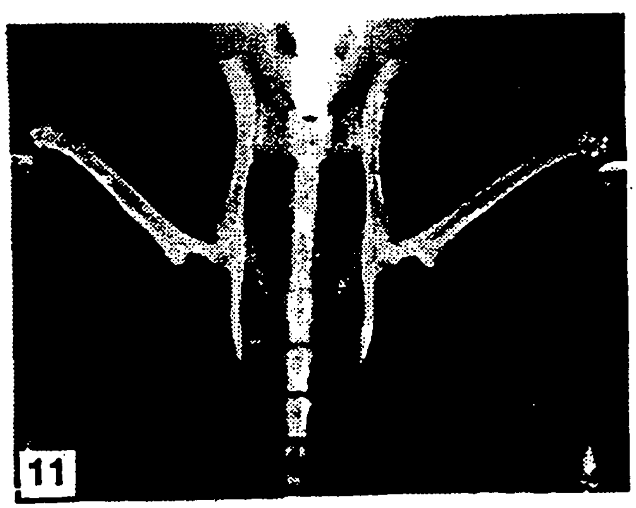


FIG.6I

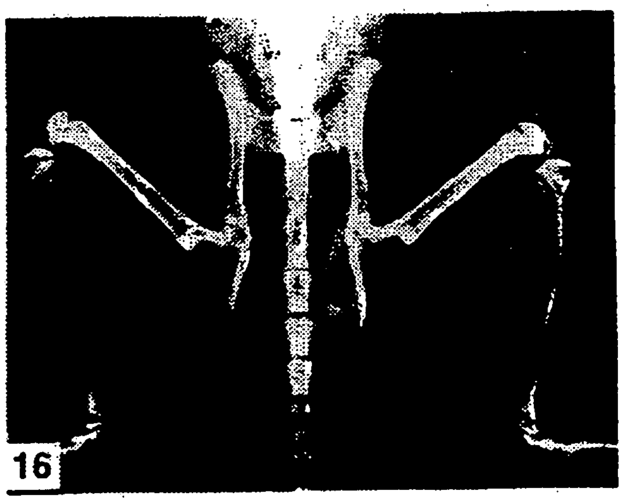


FIG.6J

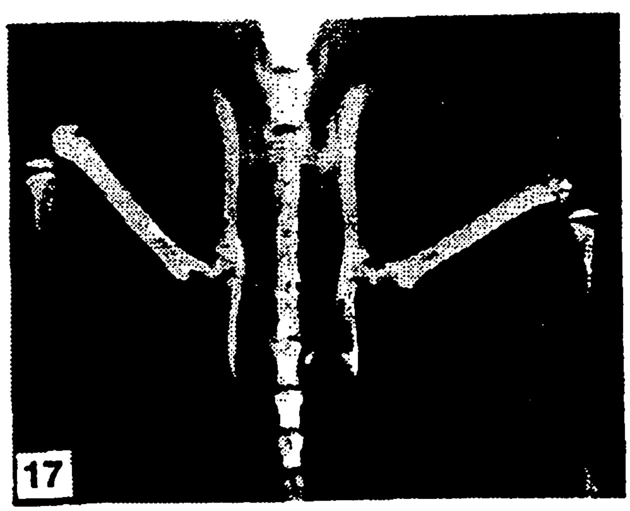


FIG.7A

FIG.7B

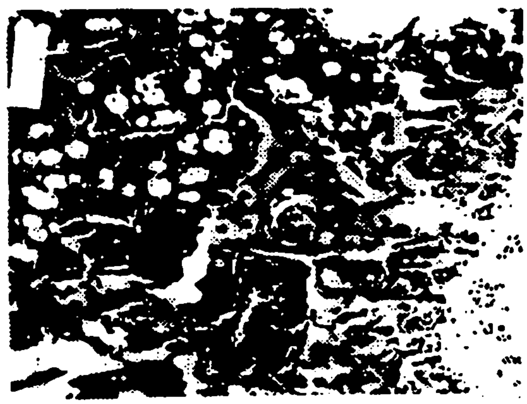


FIG.7C

FIG.7D

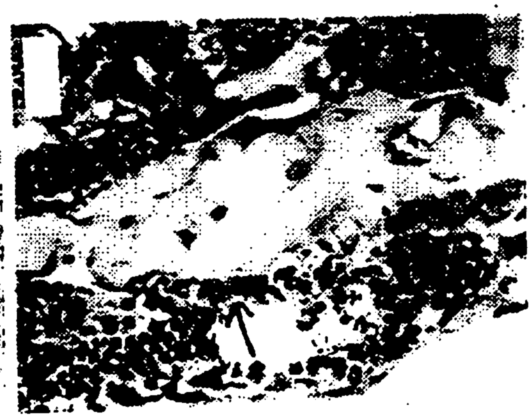


FIG.7E

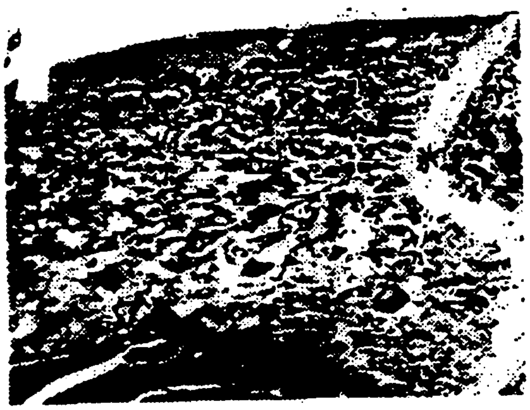


FIG.7F

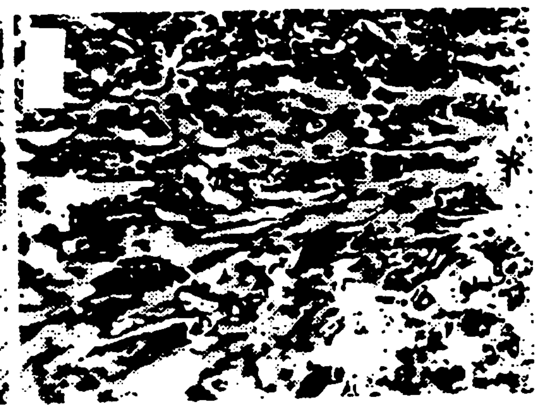


FIG.7G

FIG.7H

FIG.8A

FIG.8B

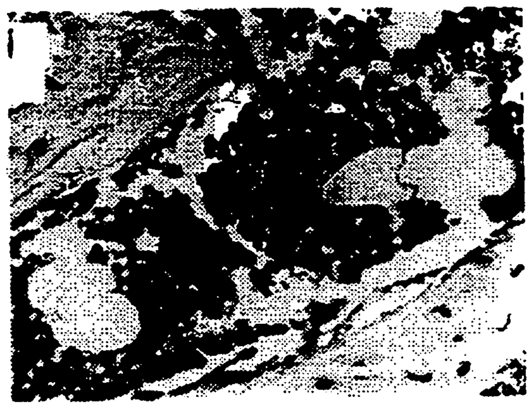


FIG.8C

FIG.8D

FIG.9A

```
       10                 30                 50
CCTTATATAARACGTCATGATTGCCTGGGCTGCAGAGACGCACCTAGCACTGACCCAGCG
       70                 90                 110
GCTGCCTCCTGAGGTTTCCCGAGGACCACAATGAACAAGTGGCTGTGCTGCGCACTCCTG
                              M  N  K  W  L  C  C  A  L  L
       130                150                170
GTGCTCCTGGACATCATTGAATGGACAACCCAGGAAACCCTTCCTCCAAAGTACTTGCAT
V  L  L  D  I  I  E  W  T  T  Q  E  T  L  P  P  K  Y  L  H
       190                210                230
TATGACCCAGAAACTGGTCATCAGCTCCTGTGTGACAAATGTGCTCCTGGCACCTACCTA
Y  D  P  E  T  G  H  Q  L  L  C  D  K  C  A  P  G  T  Y  L
       250                270                290
AAACAGCACTGCACAGTGAGGAGGAAGACATTGTGTGTCCCTTGCCCTGACCACTCTTAT
K  Q  H  C  T  V  R  R  K  T  L  C  V  P  C  P  D  H  S  Y
       310                330                350
ACGGACAGCTGGCACACCAGTGATGAGTGTGTGTATTGCAGCCCAGTGTGCAAGGAACTG
T  D  S  W  H  T  S  D  E  C  V  Y  C  S  P  V  C  K  E  L
       370                390                410
CAGTCCGTGAAGCAGGAGTGCAACCGCACCCACAACCGAGTGTGTGAGTGTGAGGAAGGG
Q  S  V  K  Q  E  C  N  R  T  H  N  R  V  C  E  C  E  E  G
       430                450                470
CGTTACCTGGAGATCGAATTCTGCTTGAAGCACCGGAGCTGTCCCCCGGGCTCCGGCGTG
R  Y  L  E  I  E  F  C  L  K  H  R  S  C  P  P  G  S  G  V
       490                510                530
GTGCAAGCTGGAACCCCAGAGCGAAACACAGTTTGCAAAAAATGTCCAGATGGGTTCTTC
V  Q  A  G  T  P  E  R  N  T  V  C  K  K  C  P  D  G  F  F
       550                570                590
TCAGGTGAGACTTCATCGAAAGCACCCTGTATAAAACACACGAACTGCAGCACATTTGGC
S  G  E  T  S  S  K  A  P  C  I  K  H  T  N  C  S  T  F  G
       610                630                650
CTCCTGCTAATTCAGAAAGGAAATGCAACACATGACAACGTGTGTTCCGGAAACAGAGAA
L  L  L  I  Q  K  G  N  A  T  H  D  N  V  C  S  G  N  R  E
       670                690                710
GCCACGCAAAAGTGTGGAATAGATGTCACCCTGTGTGAAGAGGCCTTCTTCAGGTTTGCT
A  T  Q  K  C  G  I  D  V  T  L  C  E  E  A  F  F  R  F  A
       730                750                770
GTTCCTACCAAGATTATACCAAATTGGCTGAGTGTTTTGGTGGACAGTTTGCCTGGGACC
V  P  T  K  I  I  P  N  W  L  S  V  L  V  D  S  L  P  G  T
```

FIG.9B

```
     790                 810                 830
AAAGTGAATGCCGAGAGTGTAGAGAGGATAAAACGGAGACACAGCTCACAAGAGCAAACC
K  V  N  A  E  S  V  E  R  I  K  R  R  H  S  S  Q  E  Q  T
     850                 870                 890
TTCCAGCTGCTGAAGCTGTGGAAACATCAAAACAGAGACCAGGAAATGGTGAAGAAGATC
F  Q  L  L  K  L  W  K  H  Q  N  R  D  Q  E  M  V  K  K  I
     910                 930                 950
ATCCAAGACATTGACCTCTGTGAAAGCAGCGTGCAGCGGCATCTCGGCCACTCGAACCTC
I  Q  D  I  D  L  C  E  S  S  V  Q  R  H  L  G  H  S  N  L
     970                 990                 1010
ACCACAGAGCAGCTTCTTGCCTTGATGGAGAGCCTGCCTGGGAAGAAGATCAGCCCAGAA
T  T  E  Q  L  L  A  L  M  E  S  L  P  G  K  K  I  S  P  E
     1030                1050                1070
GAGATTGAGAGAACGAGAAAGACCTGCAAATCGAGCGAGCAGCTCCTGAAGCTACTCAGT
E  I  E  R  T  R  K  T  C  K  S  S  E  Q  L  L  K  L  L  S
     1090                1110                1130
TTATGGAGGATCAAAAATGGTGACCAAGACACCTTGAAGGGCCTGATGTATGCCCTCAAG
L  W  R  I  K  N  G  D  Q  D  T  L  K  G  L  M  Y  A  L  K
     1150                1170                1190
CACTTGAAAACATCCCACTTTCCCAAAACTGTCACCCACAGTCTGAGGAAGACCATGAGG
H  L  K  T  S  H  F  P  K  T  V  T  H  S  L  R  K  T  M  R
     1210                1230                1250
TTCCTGCACAGCTTCACAATGTACAGACTGTATCAGAAGCTCTTTTTAGAAATGATAGGG
F  L  H  S  F  T  M  Y  R  L  Y  Q  K  L  F  L  E  M  I  G
     1270                1290                1310
AATCAGGTTCAATCCGTGAAAATAAGCTGCTTATAACTAGGAATGGTCACTGGGCTGTTT
N  Q  V  Q  S  V  K  I  S  C  L

CTTCA
```

FIG.9C

```
        10                            30                            50
GTATATATAACGTGATGAGCGTACGGGTGCGGAGACGCACCGGAGCGCTCGCCCAGCCGC
        70                            90                           110
CGYCTCCAAGCCCCTGAGGTTTCCGGGGACCACAATGAACAAGTTGCTGTGCTGCGCGCT
                                     M  N  K  L  L  C  C  A  L
       130                           150                           170
CGTGTTTCTGGACATCTCCATTAAGTGGACCACCCAGGAAACGTTTCCTCCAAAGTACCT
 V  F  L  D  I  S  I  K  W  T  T  Q  E  T  F  P  P  K  Y  L
       190                           210                           230
TCATTATGACGAAGAAACCTCTCATCAGCTGTTGTGTGACAAATGTCCTCCTGGTACCTA
 H  Y  D  E  E  T  S  H  Q  L  L  C  D  K  C  P  P  G  T  Y
       250                           270                           290
CCTAAAACAACACTGTACAGCAAAGTGGAAGACCGTGTGCGCCCCTTGCCCTGACCACTA
 L  K  Q  H  C  T  A  K  W  K  T  V  C  A  P  C  P  D  H  Y
       310                           330                           350
CTACACAGACAGCTGGCACACCAGTGACGAGTGTCTATACTGCAGCCCCGTGTGCAAGGA
 Y  T  D  S  W  H  T  S  D  E  C  L  Y  C  S  P  V  C  K  E
       370                           390                           410
GCTGCAGTACGTCAAGCAGGAGTGCAATCGCACCCACAACCGCGTGTGCGAATGCAAGGA
 L  Q  Y  V  K  Q  E  C  N  R  T  H  N  R  V  C  E  C  K  E
       430                           450                           470
AGGGCGCTACCTTGAGATAGAGTTCTGCTTGAAACATAGGAGCTGCCCTCCTGGATTTGG
 G  R  Y  L  E  I  E  F  C  L  K  H  R  S  C  P  P  G  F  G
       490                           510                           530
AGTGGTGCAAGCTGGAACCCCAGAGCGAAATACAGTTTGCAAAAGATGTCCAGATGGGTT
 V  V  Q  A  G  T  P  E  R  N  T  V  C  K  R  C  P  D  G  F
       550                           570                           590
CTTCTCAAATGAGACGTCATCTAAAGCACCCTGTAGAAAACACACAAATTGCAGTGTCTT
 F  S  N  E  T  S  S  K  A  P  C  R  K  H  T  N  C  S  V  F
       610                           630                           650
TGGTCTCCTGCTAACTCAGAAAGGAAATGCAACACACGACAACATATGTTCCGGAAACAG
 G  L  L  L  T  Q  K  G  N  A  T  H  D  N  I  C  S  G  N  S
       670                           690                           710
TGAATCAACTCAAAAATGTGGAATAGATGTTACCCTGTGTGAGGAGGCATTCTTCAGGTT
 E  S  T  Q  K  C  G  I  D  V  T  L  C  E  E  A  F  F  R  F
       730                           750                           770
TGCTGTTCCTACAAAGTTTACGCCTAACTGGCTTAGTGTCTTGGTAGACAATTTGCCTGG
 A  V  P  T  K  F  T  P  N  W  L  S  V  L  V  D  N  L  P  G
```

FIG.9D

```
          790                                   810                                   830
CACCAAAGTAAACGCAGAGAGTGTAGAGAGGATAAAACGGCAACACAGCTCACAAGAACA
 T  K  V  N  A  E  S  V  E  R  I  K  R  Q  H  S  S  Q  E  Q
          850                                   870                                   890
GACTTTCCAGCTGCTGAAGTTATGGAAACATCAAAACAAAGACCAAGATATAGTCAAGAA
 T  F  Q  L  L  K  L  W  K  H  Q  N  K  D  Q  D  I  V  K  K
          910                                   930                                   950
GATCATCCAAGATATTGACCTCTGTGAAAACAGCGTGCAGCGGCACATTGGACATGCTAA
 I  I  Q  D  I  D  L  C  E  N  S  V  Q  R  H  I  G  H  A  N
          970                                   990                                  1010
CCTCACCTTCGAGCAGCTTCGTAGCTTGATGGAAAGCTTACCGGGAAAGAAAGTGGGAGC
 L  T  F  E  Q  L  R  S  L  M  E  S  L  P  G  K  K  V  G  A
         1030                                  1050                                  1070
AGAAGACATTGAAAAAACAATAAAGGCATGCAAACCCAGTGACCAGATCCTGAAGCTGCT
 E  D  I  E  K  T  I  K  A  C  K  P  S  D  Q  I  L  K  L  L
         1090                                  1110                                  1130
CAGTTTGTGGCGAATAAAAAATGGCGACCAAGACACCTTGAAGGGCCTAATGCACGCACT
 S  L  W  R  I  K  N  G  D  Q  D  T  L  K  G  L  M  H  A  L
         1150                                  1170                                  1190
AAAGCACTCAAAGACGTACCACTTTCCCAAAACTGTCACTCAGAGTCTAAAGAAGACCAT
 K  H  S  K  T  Y  H  F  P  K  T  V  T  Q  S  L  K  K  T  I
         1210                                  1230                                  1250
CAGGTTCCTTCACAGCTTCACAATGTACAAATTGTATCAGAAGTTATTTTTAGAAATGAT
 R  F  L  H  S  F  T  M  Y  K  L  Y  Q  K  L  F  L  E  M  I
         1270                                  1290                                  1310
AGGTAACCAGGTCCAATCAGTAAAAATAAGCTGCTTATAACTGGAAATGGCCATTGAGCT
 G  N  Q  V  Q  S  V  K  I  S  C  L
         1330                                  1350
GTTTCCTCACAATTGGCGAGATCCCATGGATGATAA
```

FIG.9E

```
muosteo.frg  MNKWLCCALLVLLDIIEWTTQETLPPKYLHYDPETGHQLLCDKCAPGTYL   50
ratosteo.frg MNKWLCCALLVFLDIIEWTTQETFPPKYLHYDPETGRQLLCDKCAPGTYL   50
huosteo.frg  MNKLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCDKCPPGTYL   50

muosteo.frg  KQHCTVRRKTLCVPCPDHSYTDSWHTSDECVYCSPVCKELQSVKQECNRT  100
ratosteo.frg KQHCTVRRKTLCVPCPDYSYTDSWHTSDECVYCSPVCKELQTVKQECNRT  100
huosteo.frg  KQHCTAKWKTVCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRT  100

muosteo.frg  HNRVCECEEGRYLEIEFCLKHRSCPPGSGVVQAGTPERNTVCKKCPDGFF  150
ratosteo.frg HNRVCECEEGRYLELEFCLKHRSCPPGLGVLQAGTPERNTVCKRCPDGFF  150
huosteo.frg  HNRVCECKEGRYLEIEFCLKHRSCPPGFGVVQAGTPERNTVCKRCPDGFF  150

muosteo.frg  SGETSSKAPCIKHTNCSTFGLLLIQKGNATHDNVCSGNREATQKCGIDVT  200
ratosteo.frg SGETSSKAPCRKHTNCSSLGLLLIQKGNATHDNVCSGNREATQNCGIDVT  200
huosteo.frg  SNETSSKAPCRKHTNCSVFGLLLTQKGNATHDNICSGNSESTQKCGIDVT  200
```

FIG.9F

```
muosteo.frg  LCEEAFFRFAVPTKIIPNWLSVLVDSLPGTKVNAESVERIKRRHSSQEQT  250
ratoste .frg LCEEAFFRFAVPTKIIPNWLSVLVDSLPGTKVNAESVERIKRRHSSQEQT  250
huosteo.frg  LCEEAFFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSSQEQT  250

muosteo.frg  FQLLKLWKHQNRDQEMVKKIIQDIDLCESSVQRHLGHSNLTTEQLLALME  300
ratosteo.frg FQLLKLWKHQNRDQEMVKKIIQDIDLCESSVQRHIGHANLTTEQLRILME  300
huosteo.frg  FQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME  300

muosteo.frg  SLPGKKISPEEIERTRKTCKSSEQLLKLLSLWRIKNGDQDTLKGLMYALK  350
ratosteo.frg SLPGKKISPDEIERTRKTCKPSEQLLKLLSLWRIKNGDQDTLKGLMYALK  350
huosteo.frg  SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALK  350

muosteo.frg  HLKTSHFPKTVTHSLRKTMRFLHSFTMYRLYQKLFLEMIGNQVQSVKISC  400
ratosteo.frg HLKAYHFPKTVTHSLRKTIRFLHSFTMYRLYQKLFLEMIGNQVQSVKISC  400
huosteo.frg  HSKTYHFPKTVTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISC  400

muosteo.frg  L                                                   401
ratosteo.frg L                                                   401
huosteo.frg  L                                                   401
```

FIG.10

```
ltnrr   C P Q - G K Y I H P Q N N S I C C T K C H K G T Y L Y N D C P G P G Q D T D C R E C E S G S F T A S   49
humoste P P K Y L H Y D E E T S H Q L L C D K C P P G T Y L K Q H C T A K - W K T V C A P C P D H Y Y T D S   49

ltnrr   E N H L R H C L S C S - K C R K E M G Q V E I S S C T V D R D T V C G C R K N Q Y R H Y W S E N L F   98
humoste W H T S D E C L Y C S P V C - K E L Q Y V K - Q E C N R T H N R V C E C K E G R Y L E I - - - E - F   93

ltnrr   Q C F N C S L C L N G - T V H L S C Q E K Q N T V C T - C H A G F F L R E - - - - N E C V S C   139
humoste - C L K H R S C P P G F G V V Q A G T P E R N T V C K R C P D G F F S N E T S S K A P C R K H   139
```

FIG.11

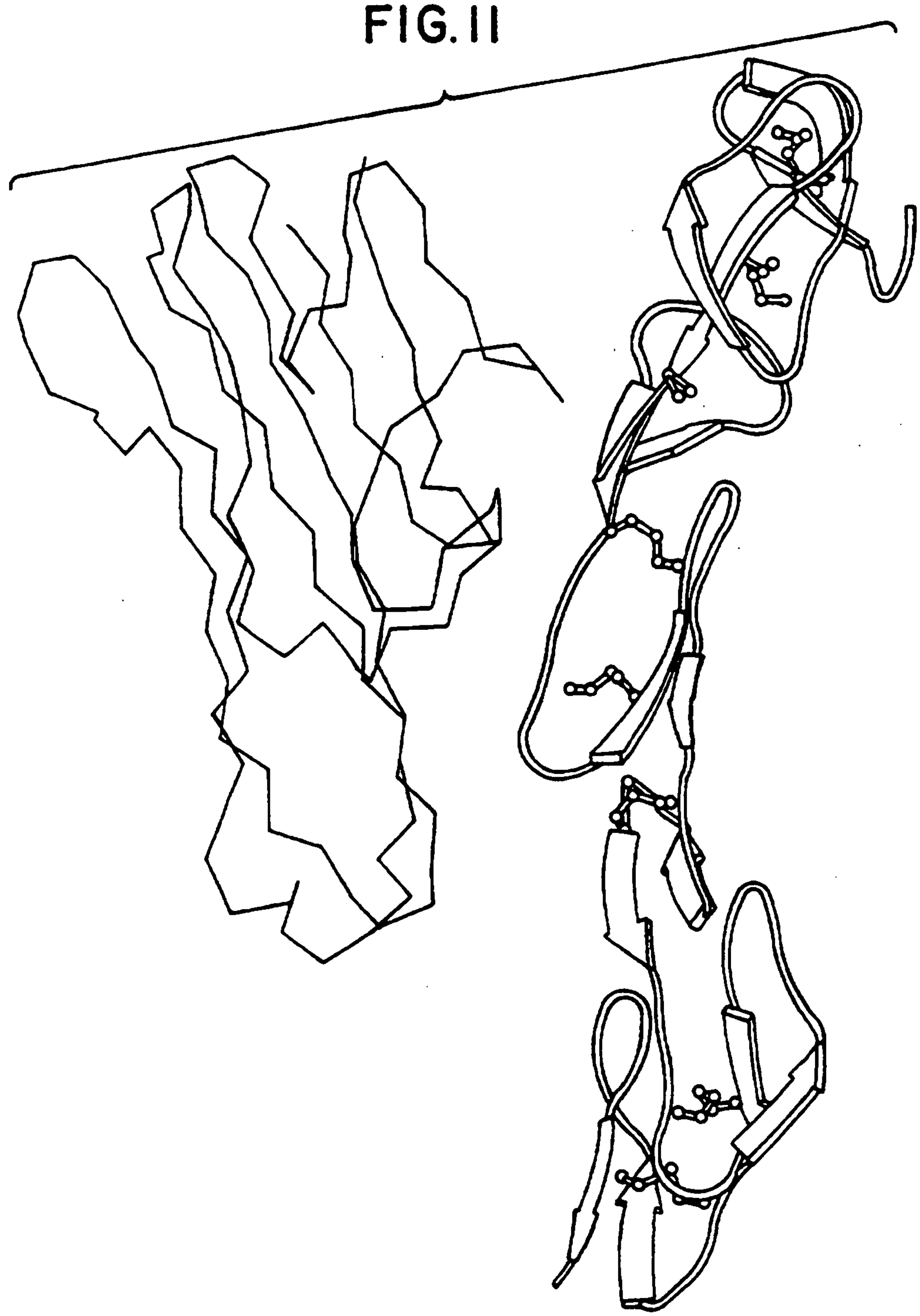


FIG. 12A

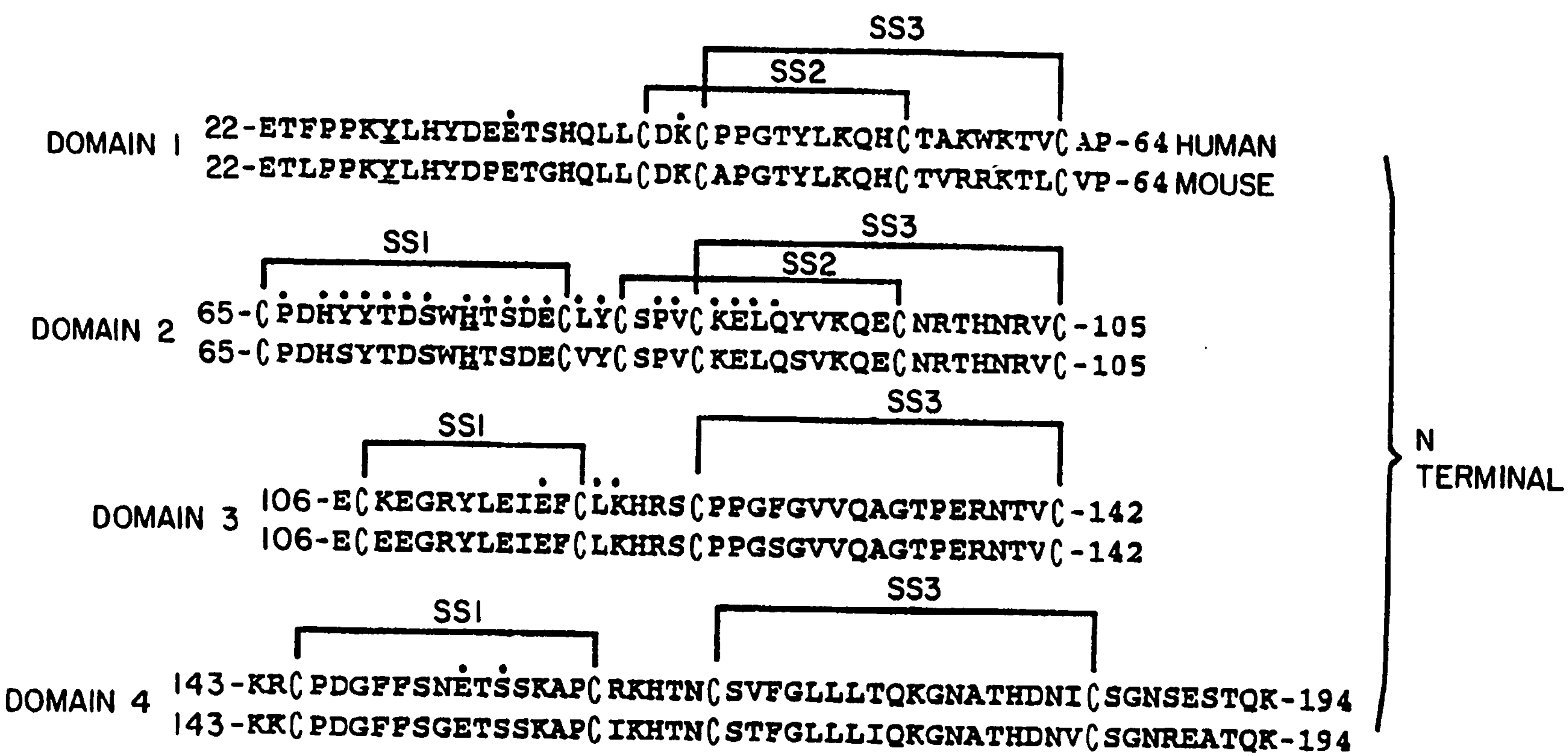

SS3
SS2
DOMAIN 1
22-ETFPPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKTVCAP-64 HUMAN
22-ETLPPKYLHYDPETGHQLLCDKCAPGTYLKQHCTVRRKTLCVP-64 MOUSE
SSI
SS3
SS2
DOMAIN 2
65-CPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVC-105
65-CPDHSYTDSWHTSDECVYCSPVCKELQSVKQECNRTHNRVC-105
SSI
SS3
DOMAIN 3
106-ECKEGRYLEIEFCLKHRSCPPGFGVVQAGTPERNTVC-142
106-ECEEGRYLEIEFCLKHRSCPPGSGVVQAGTPERNTVC-142
SSI
SS3
DOMAIN 4
143-KRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNATHDNICSGNSESTQK-194
143-KKCPDGFFSGETSSKAPCIKHTNCSTFGLLLIQKGNATHDNVCSGNREATQK-194
N
TERMINAL

FIG.12B

195-CGIDVTLCEEAFFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSS-246
195-CGIDVTLCEEAFFRFAVPTKIIPNWLSVLVDSLPGTKVNAESVERIKRRHSS-246

247-QEQTFQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSL-298
247-QEQTFQLLKLWKHQNRDQEMVKKIIQDIDLCESSVQRHLGHSNLTTEQLLAL-298

299-MESLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALK-350
299-MESLPGKKISPEEIERTRKTCKSSEQLLKLLSLWRIKNGDQDTLKGLMYALK-350

351-HSKTYHFPKTVTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL-401
351-HLKTSHFPKTVTHSLRKTMRFLHSFTMYRLYQKLFLEMIGNQVQSVKISCL-401

C TERMINAL

FIG.13A

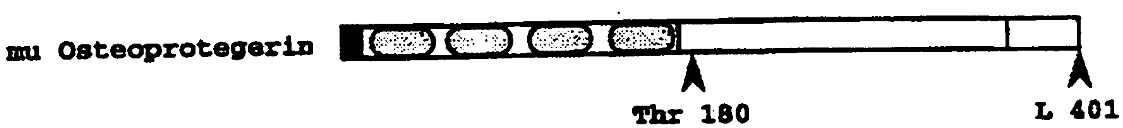


FIG.13B

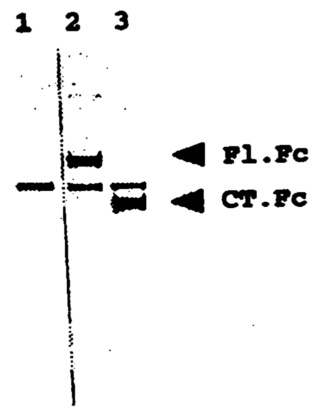


FIG.13C

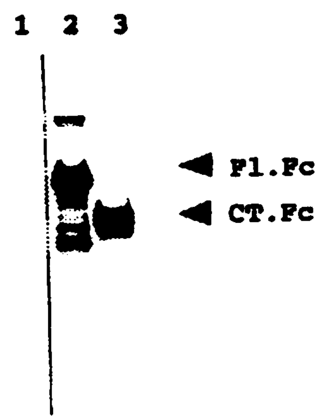


FIG.14A

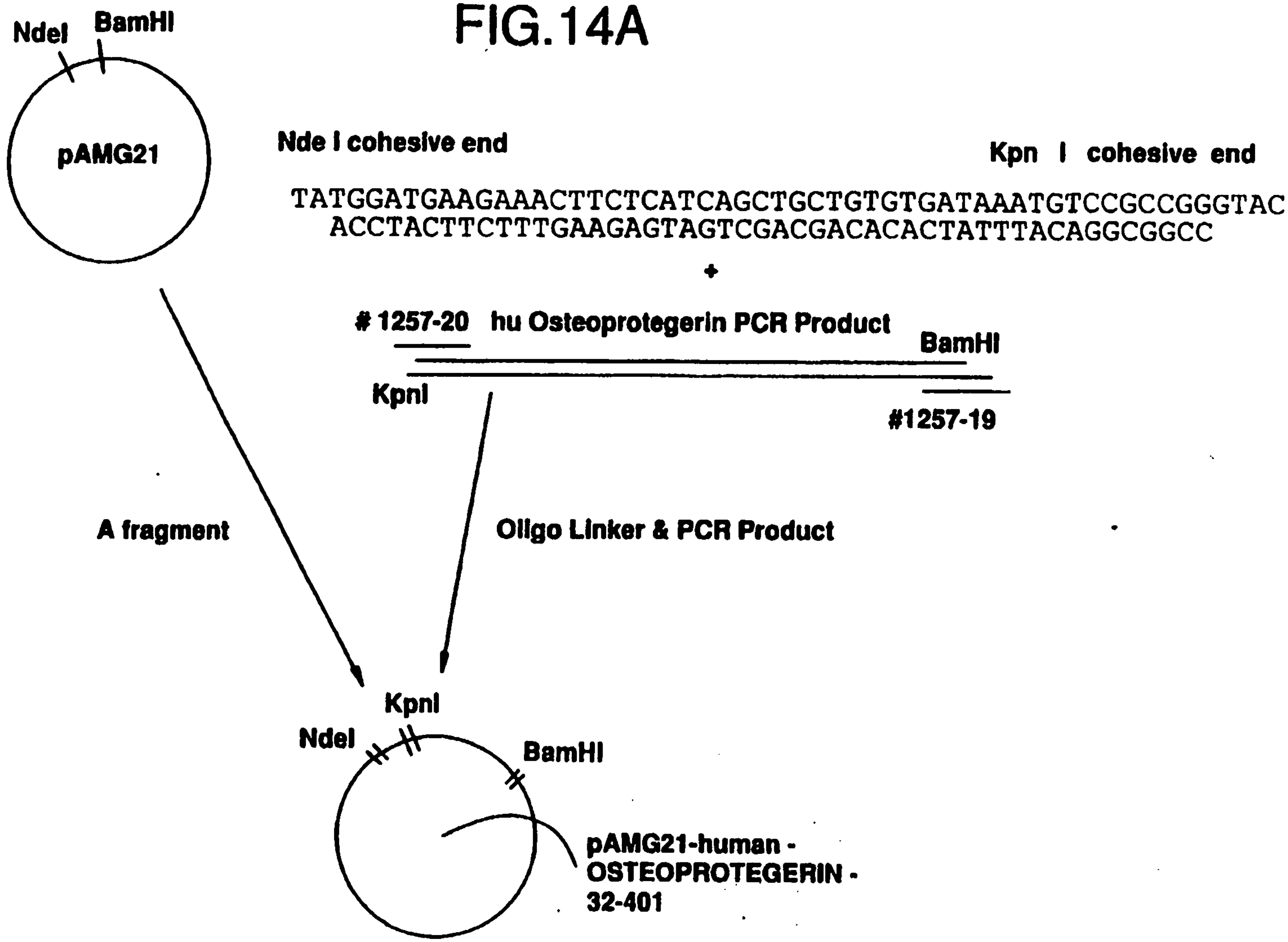

NdeI
BamHI
pAMG21
Nde I cohesive end
Kpn I cohesive end
TATGGATGAAGAAACTTCTCATCAGCTGCTGTGTGATAAATGTCCGCCGGGTAC
ACCTACTTCTTTGAAGAGTAGTCGACGACACACTATTTACAGGCGGCC
+
1257-20
hu Osteoprotegerin PCR Product
BamHI
KpnI
#1257-19
A fragment
Oligo Linker & PCR Product
KpnI
NdeI
BamHI
pAMG21-human -
OSTEOPROTEGERIN -
32-401

FIG. 14B

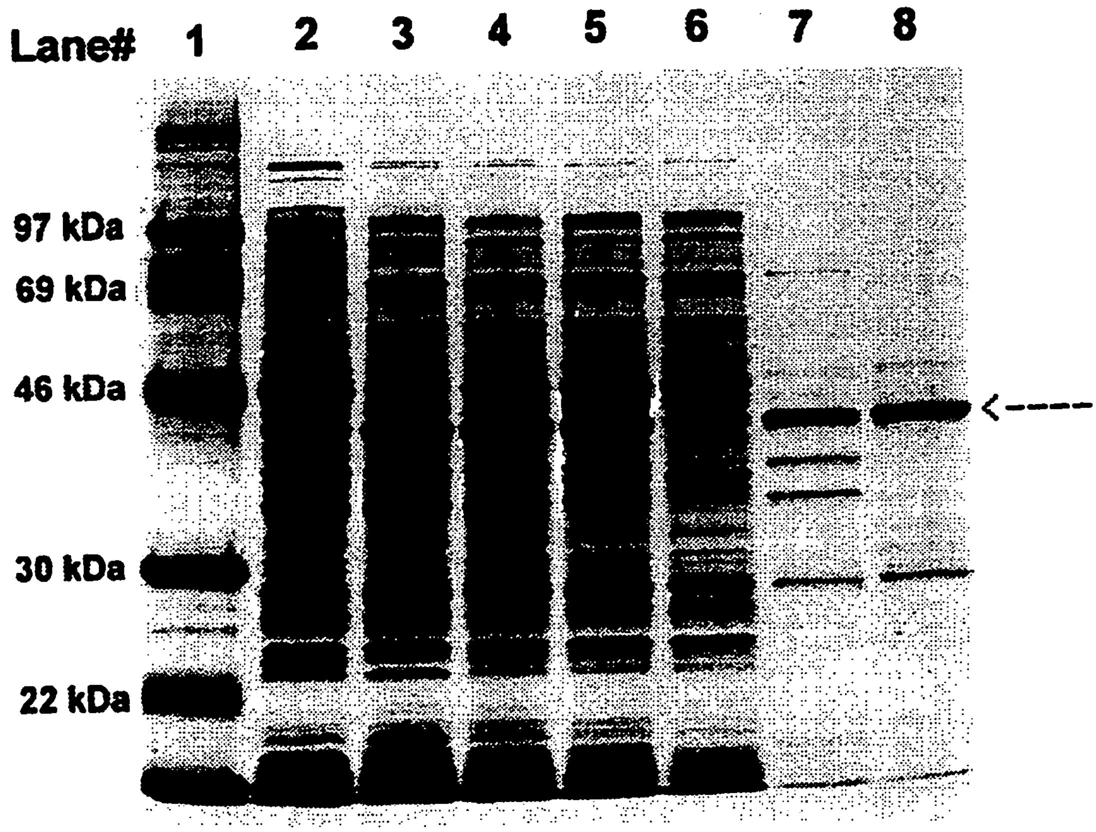


FIG.15

FIG.16A

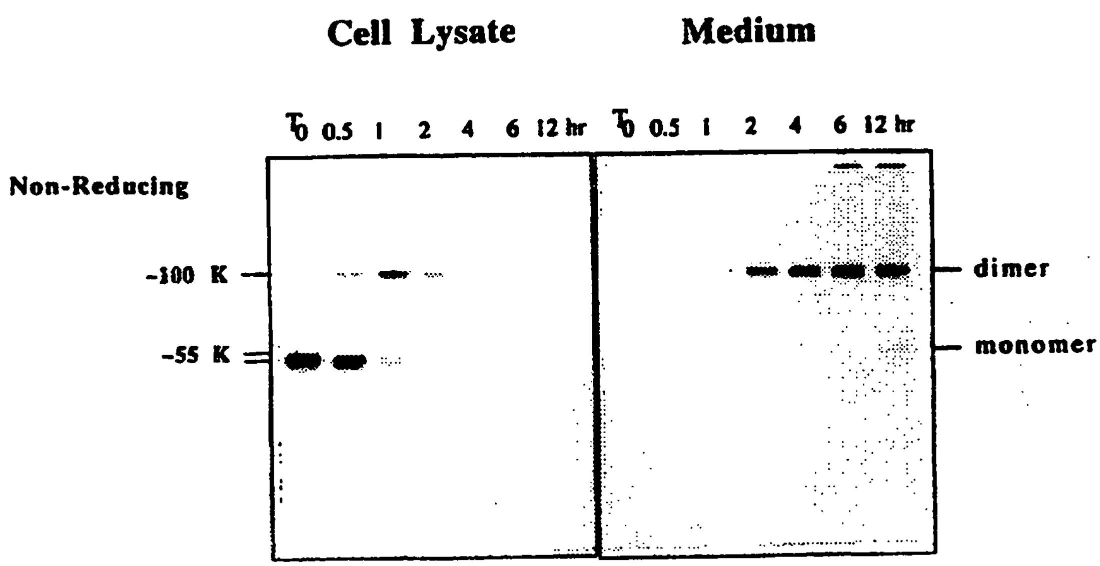


FIG.16B

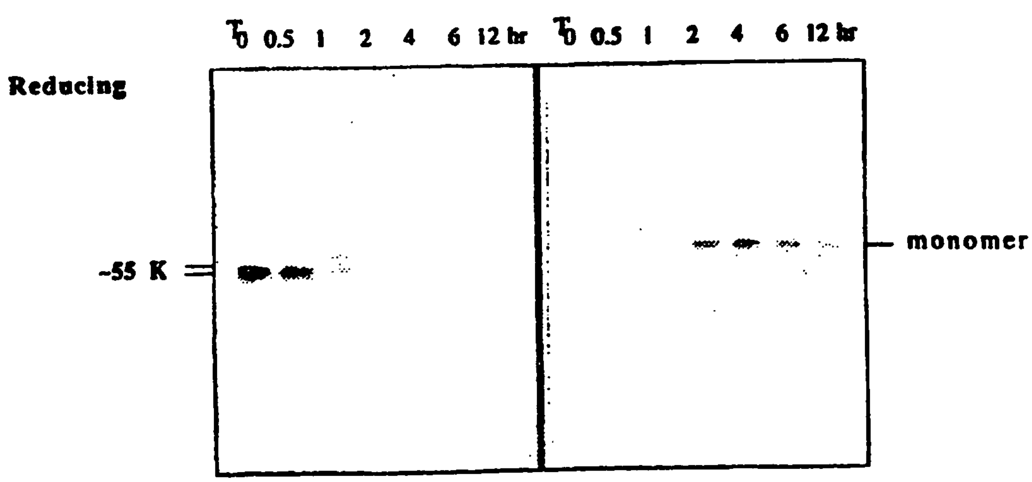


FIG.17

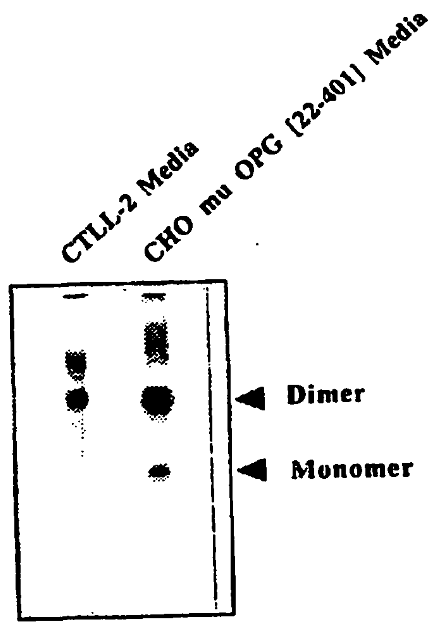
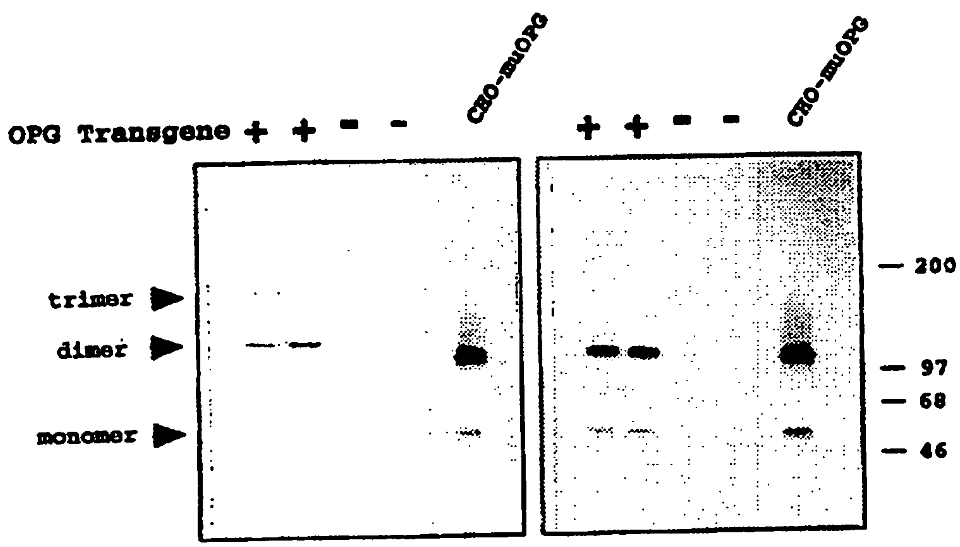


FIG.18


Serum
Liver
OPG Transgene + + - -
CHO-muOPG
+ + - -
CHO-muOPG
trimer
dimer
monomer
200
97
68
46

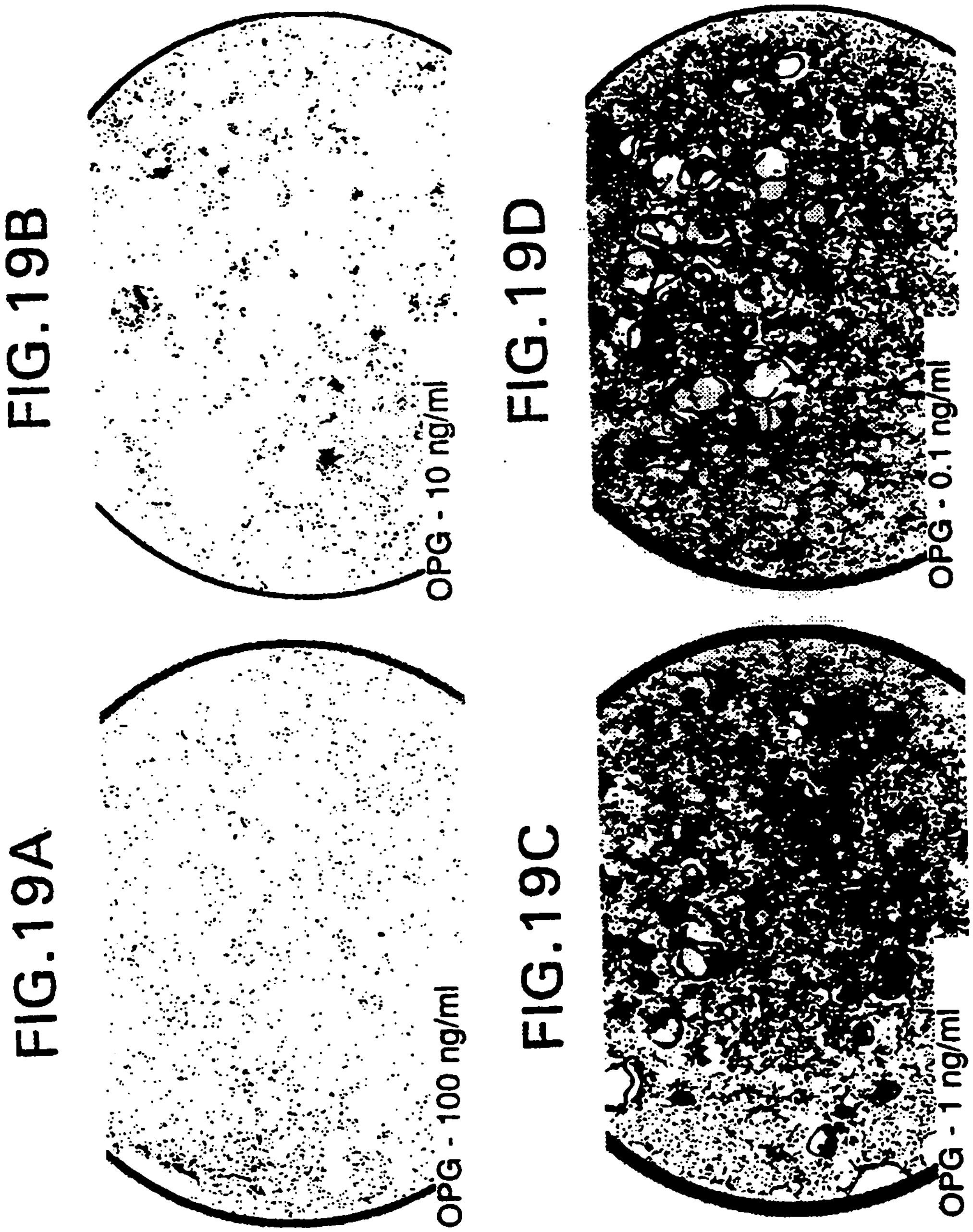


FIG.19A

FIG.19B

FIG.19C

FIG.19D

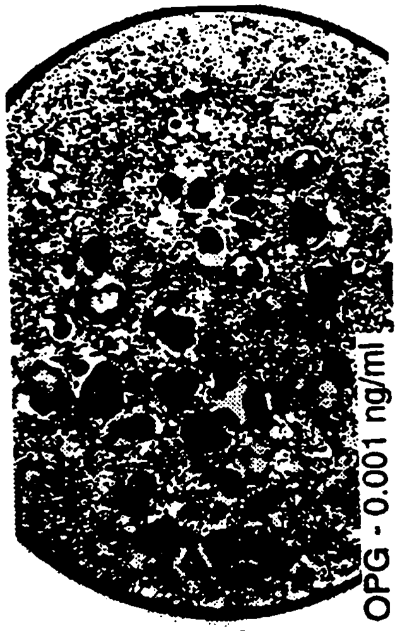

OPG - 0.001 ng/ml

FIG.19F

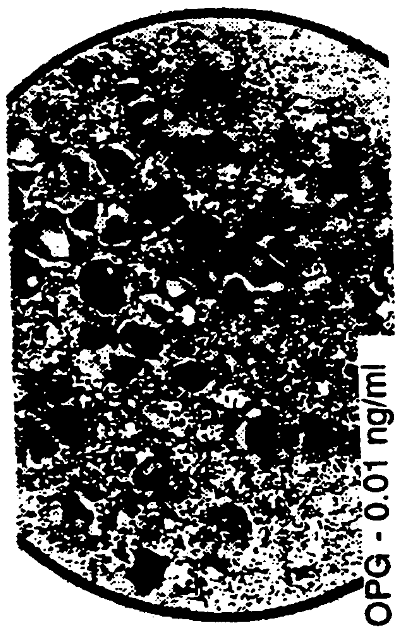

OPG - 0.01 ng/ml

FIG.19E


Control

FIG.19G

FIG.20

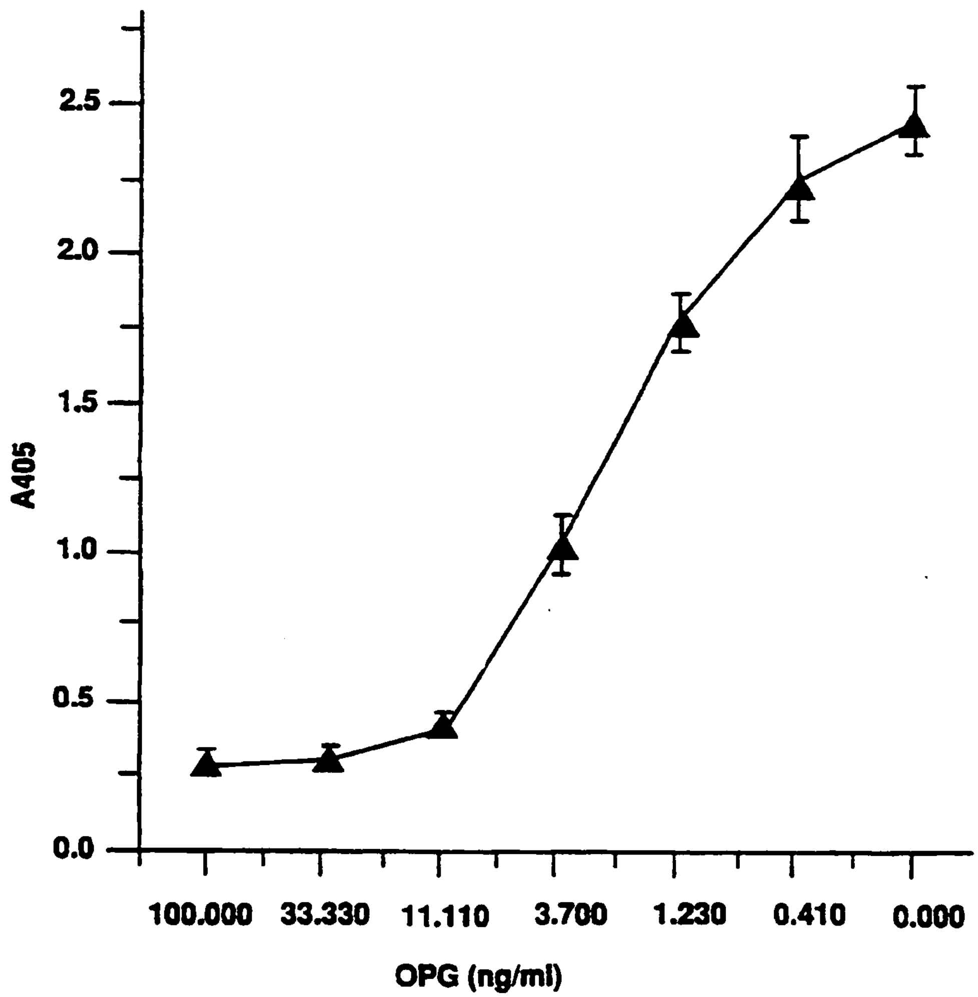
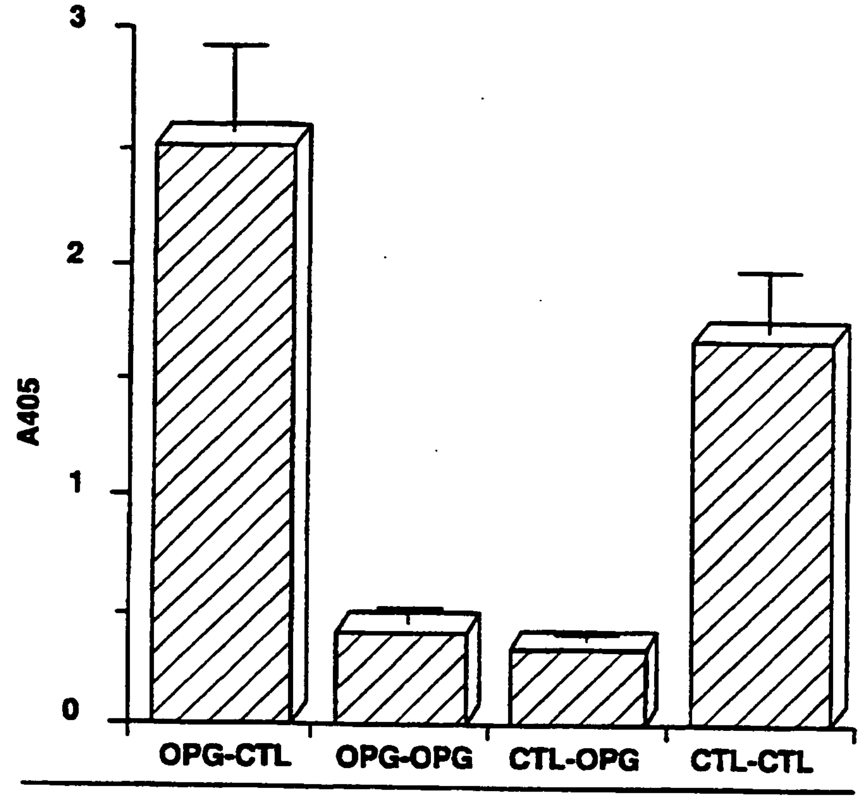

2.5
2.0
1.5
1.0
0.5
0.0
A405
100.000
33.330
11.110
3.700
1.230
0.410
0.000
OPG (ng/ml)

FIG.21



Legend

Growth	Intermediate	Terminal
Bone marrow cells CSF -1	PGE2 + CSF-1	ST2 cells 1,25 (OH)2 D3 Dexamethasone
4 days	2 days	8 - 10 days

Groups	OPG	OPG
CTL - CTL	— — —	— — —
OPG - CTL	100 ng/ml	— — —
OPG - OPG	— — —	100 ng/ml
OPG - OPG	100 ng/ml	100 ng/ml

FIG.22A

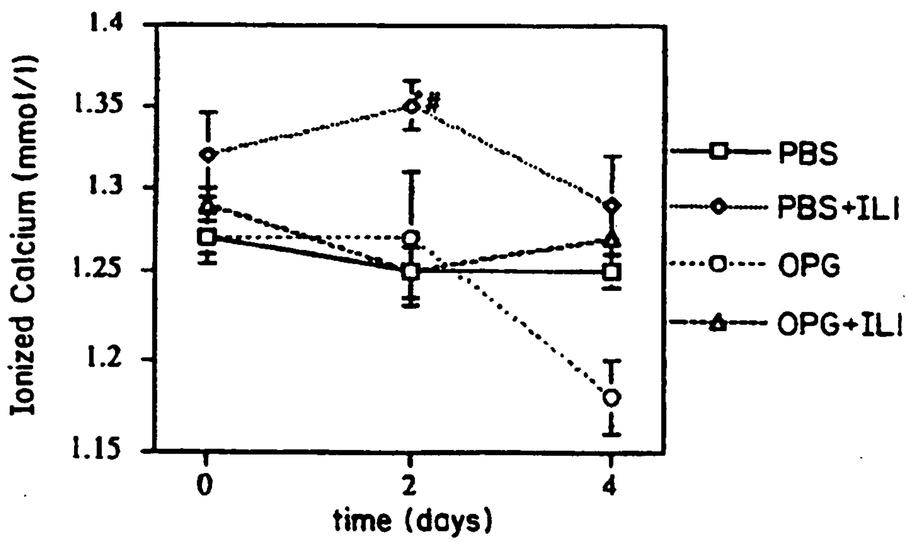
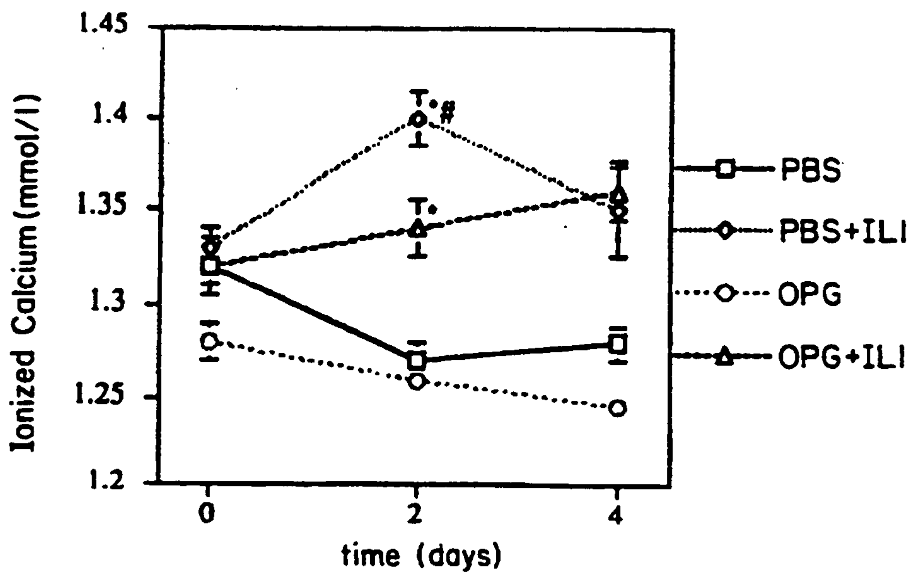


FIG.22B



* Different to PBS, $p < 0.05$
# Different to OPG + IL1, $p < 0.05$

FIG.23A

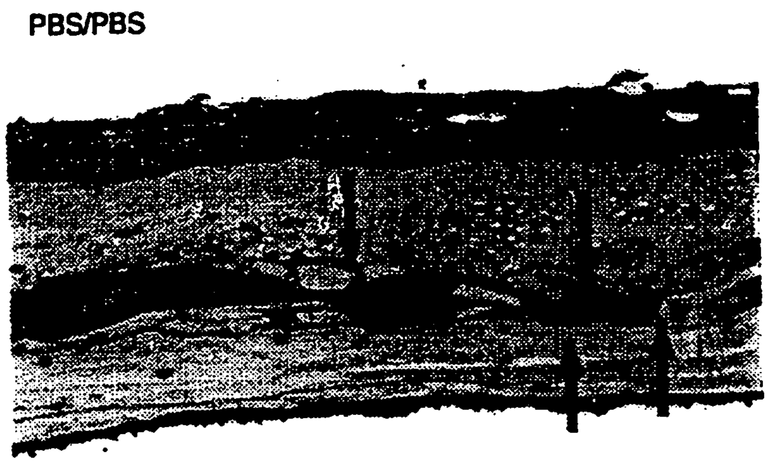


FIG.23B

FIG.23C

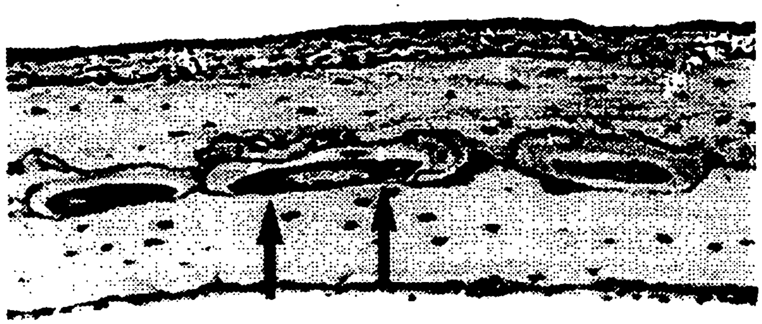
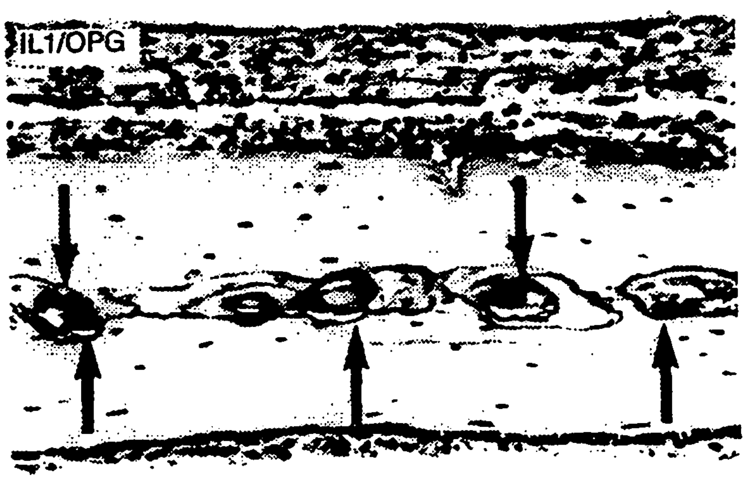


FIG.23D



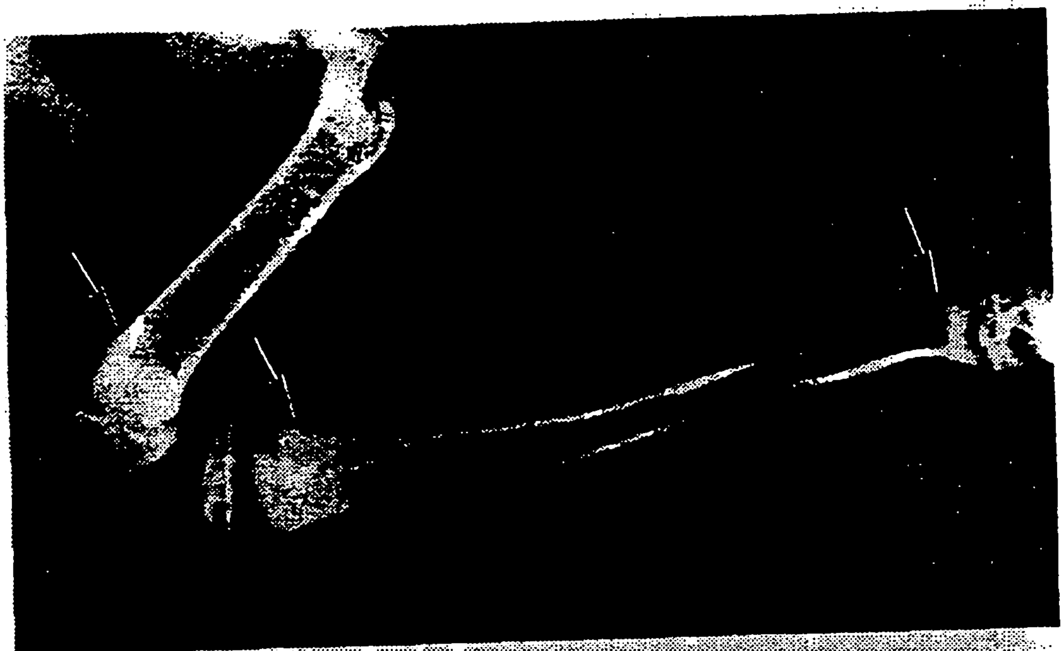
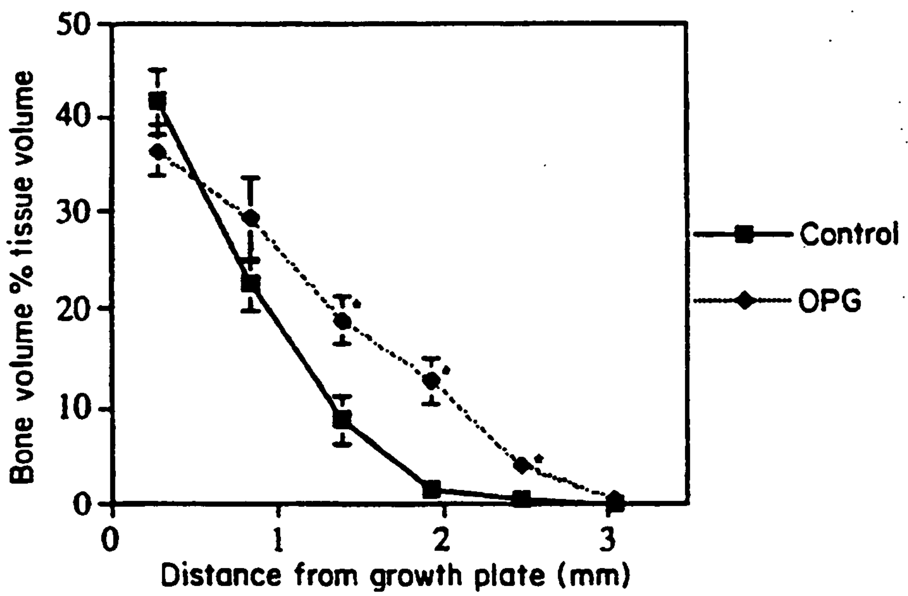


FIG.24B

FIG.24A

FIG.25



* Different to control p < 0.01

FIG.26A

FIG.26.B

FIG.27

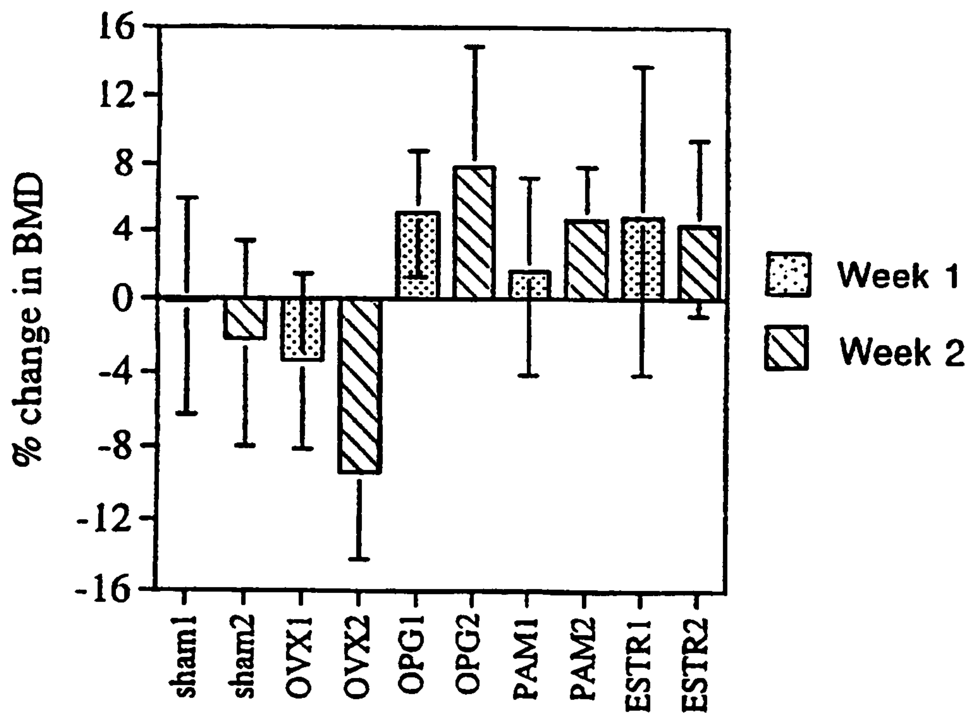
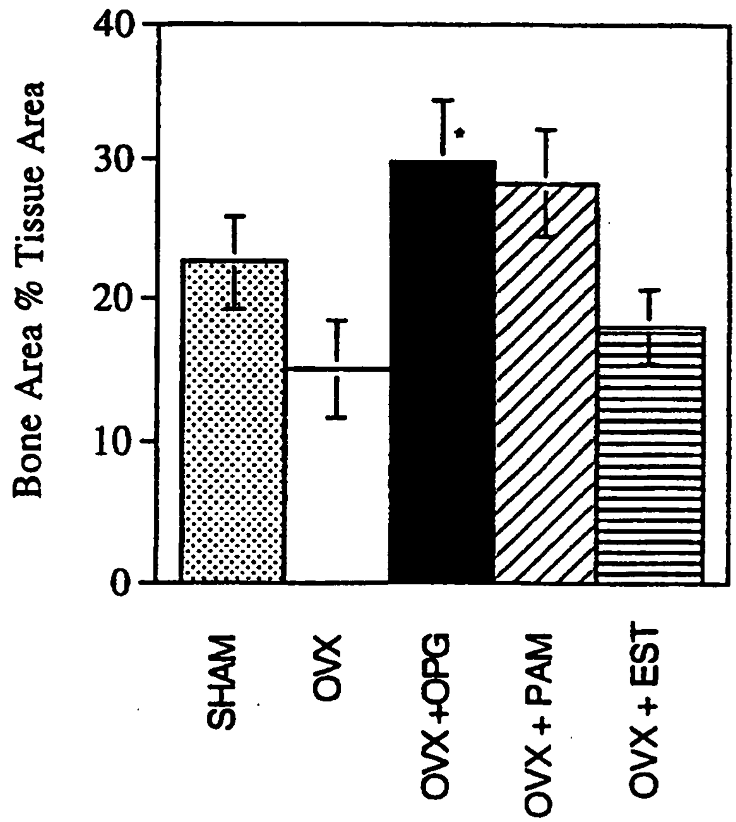

16
12
8
4
0
-4
-8
-12
-16
% change in BMD
sham1
sham2
OVX1
OVX2
OPG1
OPG2
PAM1
PAM2
ESTR1
ESTR2
Week 1
Week 2

FIG.28



* Different to OVX $p < 0.05$

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/20621

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/715 C12N5/10 A01K67/027 C07K19/00 C12N15/62 C07K16/28 C07K1/107 C12Q1/68 G01N33/50 G01N33/566 A61K38/17 A61K48/00 C12N1/21 //(C12N1/21,

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A01K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CELL, vol. 76, 25 March 1994, pages 959-962, XP002029050 SMITH C.A. ET AL.: "The TNF receptor superfamily of cellular and viral proteins: activation, costimulation and death." cited in the application see the whole document ---	1-60
A	PROC.NATL.ACAD.SCI.USA, vol. 88, 1991, pages 2830-2834, XP002029051 LEWIS M. ET AL.: "Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific." see figure 1 ---	1-60

-/--

[X] Further documents are listed in the continuation of box C.

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"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 1997	16. 04. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Mandl, B

INTERNATIONAL SEARCH REPORT

Inte   nal Application No

A. CLASSIFICATION F SUBJECT MATTER
IPC 6 C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCIENCE, vol. 252, 1991, pages 1651-1656, XP000645049 ADAMS M.D.: "Complementary DNA sequencing:expressed sequence tags and human genome project." cited in the application see the whole document ---	1-60
A	US 5 447 851 A (BEUTLER BRUCE A ET AL) 5 September 1995 see the whole document ---	36-38,57
A	US 4 179 337 A (DAVIS FRANK F ET AL) 18 December 1979 cited in the application see the whole document -----	28,29

☐ Further documents are listed in the continuation of box C.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT	:rnational application No. PCT/US 96/20621

Box I bservations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 43-45,49-53
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/20621

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5447851 A	05-09-95	NONE	
US 4179337 A	18-12-79	CA 1033673 A	27-06-78
		CH 616942 A	30-04-80
		DE 2433883 A	05-02-76
		FR 2313939 A	07-01-77
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		JP 56023587 B	01-06-81
		NL 7409770 A	22-01-75
		SE 441753 B	04-11-85
		SE 7409366 A	21-01-75

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